VISIT…

DRUGS AND THE PHARMACEUTICAL SCIENCES VOLUME 141

New Drug Development

Regulatory Paradigms for Clinical Pharmacology and Biopharmaceutics

edited by
Chandrahas G. Sahajwalla

New Drug Development

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Regulatory Paradigms for Clinical Pharmacology and Biopharmaceutics

edited by

Chandrahas G.Sahajwalla

U.S. Food and Drug Administration
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Foreword

The opportunity to contribute to people's health is a source of inspiration to those working in drug development. However, drug development is complex, costly, and fraught with uncertainty. Success demands teamwork and extensive knowledge of current technology and regulations. The discipline of clinical pharmacology has, over the years, become an important and integral part of the drug development process. Now, in the era of individualization of drug therapies, the discipline of clinical pharmacology is strategically positioned to make seminal contributions to the understanding of the sources of variability in individual drug responses.

The biomedical advances of recent years have the potential to transform the drug development process; however, this goal can only be achieved if knowledgeable people from industry, academia, and government work together as a team. It is important that scientific personnel involved in drug development have access to up-to-date information. *New Drug Development: Regulatory Paradigms for Clinical Pharmacology,* edited by Chandrahas Sahajwalla, is a timely book which combines the scientific and regulatory aspects of clinical pharmacology and biopharmaceutics in easy-to-understand chapters that cover all aspects of drug development for these disciplines. For universities offering programs in drug development, this volume fills an existing void, and further provides a quick reference guide for the industrial or academic scientist who is new in the field of drug development.

Until now there has been no specific source where a student or new investigator could find a single, comprehensive presentation of the scientific and regulatory principles necessary for filing the clinical pharmacology and biopharmaceutics section of a new drug application (NDA) or biologies

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license application (BLA). Although this information is available in a fragmentary manner in multiple places, there has been no concise reference that gives a complete overview of the scientific and regulatory perspective and paradigms for clinical pharmacology and biopharmaceutics.

New Drug Development: Regulatory Paradigms for Clinical Pharmacology is unique in that it covers the regulations governing Investigational New Drugs (IND) and NDAs, and takes the reader through the pertinent aspects of clinical pharmacology and biopharmaceutics. This book covers in-vitro studies needed to understand properties of new drug molecules including metabolism, transporters, and interaction studies. Also included are basic concepts of bioavailability and bioequivalence, specific population studies including those in disease states such as renal and hepatic impairment, biomarkers, population pharmacokinetics, exposure-response studies, drug interactions and specific scientific issues related to selected therapeutic areas. There is also very timely coverage of specific drug development issues for chiral drugs, liposomal products, counter-bioterrorism agents, and the regulation of antidotes for nerve agent poisoning. Essential elements of biopharmaceutics for new and generic drugs have also been discussed in detail.

The contributing authors are well recognized experts in their respective fields who bring experience from regulatory organizations and academia. A global perspective is provided by the participation of authors from Europe, Canada, and the United States.

Rising prescription costs worldwide call for a reduction in drug development costs whenever possible. This can be facilitated by access to good information to assist developers in reducing the number of unnecessary or poorly designed studies. *New Drug Development: Regulatory Paradigms for Clinical Pharmacology* will provide solid information to students, teachers, and new researchers alike and can also serve as a quick reference for particular aspects of clinical pharmacology and biopharmaceutics for experienced scientists.

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Preface

After graduating in pharmaceutics and joining a multinational pharmaceutical company, I quickly realized how much I need to learn about drug development and the associated regulatory process. Most pharmaceutical scientists have gained knowledge of regulatory science from practical experience. There is not a single textbook that combines scientific and regulatory principles essential to answering the clinical pharmacology and biopharmecutics questions that arise during drug development. Motivated by the lack of such a book, I compiled this text. This book is aimed at students and new scientists in the industry and government, and at encouraging universities to incorporate training for regulatory sciences in their curriculum.

This book has been divided into five parts: History and Basic Principles (Chapters 1–4); In Vitro/Pre-Clinical (Chapters 5–7); Clinical Pharmacology (Chapters 8–16); Biopharmaceutics (Chapters 17–20) and Contemporary and Special Interest Topics (Chapters 21–25).

The first part of this book introduces the reader to regulatory history, important regulations governing clinical pharmacology and biopharmaceutics portion of the new drug application, and the review process at the Food and Drug Administration (FDA). This is followed by a part in-vitro and preclinical studies such as metabolism, drug-drug interactions, transporters and interspecies scaling. Part III introduces the reader to clinical pharmacology studies that are generally conducted. This part starts with a chapter on analytical method validation, and takes the reader through characterization of basic pharmacokinetics properties to surrogate markers, population PK and PD studies, and assessment of in-vivo drug interactions. Three chapters in this part discuss special populations like

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disease state for example (renal and hepatic impairment), gender, race, age (elderly and pediatric), pregnancy, and lactation. The last chapter in Part III discusses clinical pharmacology issues related to several specific drug classes.

Clinical pharmacology is followed by a part on biopharmaceutics. This part starts off with a chapter on bioavailability and bioequivalence (BA/BE) assessments for new and generic drugs followed by chapters on oral extended release products, and when and how one can obtain a waiver for conducting in-vivo BE studies. The last chapter in this part describes the assessment of BE of drugs administered via routes other than oral.

There are certain situations in drug development which require additional consideration. For example, the development of a chiral drug, liposomal drug product, or drugs to treat situations/illnesses created by biological and nerve poisoning agents. The last part of this book discusses such contemporary or special topics. The last chapter in this book is a tutorial in conducting statistical analysis of BE studies.

The FDA and other regulatory agencies continue to release guidances on contemporary topics. For example, when this book went in to print, guidances on pharmacogenomics/pharmacogenetics and assessment of QTc prolongation by drugs were still being developed. This book is by no means exhaustive and the reader is encouraged to refer to the regulatory agency websites on these ever-evolving contemporary topics.

The chapters in this book are the result of expertise and time devoted by many experts from the FDA and other regulatory agencies. In addition to the scientific principles, the authors were encouraged to include key points from the latest regulatory guidances. Further, authors have attempted to include the regulatory requirements from other (European, Canada) agencies and also incorporate ICH (International Conference on Harmonization) requirements. There are 25 chapters written by 40 authors in this book. I have made every attempt to use the same format and terminology and avoid duplication of information. However, since this book is aimed to be used as a teaching tool, some duplicated information was deliberately left untouched for the sake of completeness of a chapter.

This book is intended to serve as an introductory reference text to the pharmaceutical scientist, student, and researcher involved in the new drug development. This book is not intended to be used as a template, but gives the reader basic understanding of the drug development process for a new chemical being developed as a drug.

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Chandrahas Sahajwalla

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New Drug Development

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1

Introduction to Drug Development and Regulatory Decision-Making

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The science of contemporary drug development is a tremendously complex and costly process but it has successfully advanced our understanding of modern diseases and has improved public health significantly by providing society with many valuable drug treatments. A crucial step in the drug development process is the submission of nonclinical and clinical data and information in a New Drug Application (NDA) to the Food and Drug Administration (FDA) by a sponsor seeking marketing authorization. A typical new molecular entity (NME) that is the subject of a NDA has most likely been studied preclinically for 5–7 years and has been in clinical trials for 6–7 years. The average cost of bringing an NME to market is somewhere between 500 and 800 million dollars including the costs of lost opportunities and lead-compound failures [1]. With this investment of time and money, many scientists involved in drug development have explored various ways to make drug development as efficient, and yet informative, as possible [2].

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Despite its successes, the drug development process, including regulatory decision-making based on benefit/risk assessments, can be improved in three areas.

1. Provide a greater understanding of human health and the causes of diseases at a genomic or molecular level. This would address the well-known heterogeneity of disease states that underlies the wide interindividual variation in efficacy observed with many common treatments. For example, incomplete or absence of response occurs in 30–50% of eligible patients with hypercholesteremia who are treated with "statins." With greater insights into health and disease, sponsors would be more likely to identify a target protein or receptor and to find the best NME to provide preventive, curative, or palliative treatment for patients.
2. Improve the safety of medicines. Adverse drug reactions (ADRs) have had a major impact on morbidity, mortality, and health economics. In studies going back to 1974, up to the present time, approximately 15–20% of hospitalized children and 25–30% of hospitalized adults have experienced drug-related adverse events [3, 4]. The overall incidence of drug-induced adverse events in nonhospitalized patients is thought to be around 7% [5]. The economic cost of drug-related morbidity and mortality to society has been estimated to be almost 200 billion dollars [6]. While there are many reasons, some of them unknown, for the relatively high incidence of ADRs (e.g., medication errors, drug interactions), it is thought that the majority of the risks associated with drug therapy are known and most drug-related adverse events are preventable [7].
3. Optimize drug doses and dosing schedules. Approximately 70% of drug-related adverse events are due to extended pharmacological actions. Thus, there is growing evidence to suggest that drug doses approved for marketing may be higher than is necessary and may be contributing to the high frequency of serious drug side effects. A recent study that examined the doses of 354 prescription drugs recommended in the label and released between 1980 and 1999 found that approximately 17% of these drugs had a reduction in dose or a new restriction for use in special populations such as patients with renal or hepatic disease [8]. Furthermore, it has been reported that prescribers in their practice frequently use doses which are lower than the FDA-approved label dose [9]. In an informal survey, it was also found that doses approved in other countries, e.g., Japan, are

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lower than those approved in the United States and most often there are no apparent scientific rationale for these differences.

These three areas of improvement should be viewed as a challenge to the scientific community in industry, academia, and the regulatory agencies to engage in dialogue and scientific collaboration to optimize the drug development process. This is especially important in light of the emergence of new genetic technologies and our understanding of the human genome that provides us new ways to ask important questions during the drug development process. Indeed, the promise of personalized or predictive medicine that stems from pharmacogenetics and pharmacogenomics means that the benefit/risk ratio of drugs is systematically optimized by identifying and selecting the right drug target, developing the right drug, and delivering the right dose to the right patient.

ROLE OF CLINICAL PHARMACOLOGY

At the core of the drug development process is a fundamental understanding of the clinical pharmacology of the drug substance. Clinical pharmacology can be thought of as a translational science in which basic information about the relationship between a drug's dose, local or systemic exposure and response (related to either efficacy or safety) is applied in the context of patient care. Knowledge of this relationship, which is a key to successful therapeutics, and how it is altered by the intrinsic (age, gender, renal function, etc.) and extrinsic (diet, drugs, life-style) factors of an individual patient is one of the major contributions of clinical pharmacology to drug development and regulatory decision-making.

Once a lead compound with the intended pharmacological action is identified, the step-wise process to characterize and potentially optimize its pharmacokinetic (PK) properties (i.e., absorption, distribution, metabolism, and excretion), as well as to minimize its pharmacokinetic limitations (e.g., poor absorption), begins in humans as part of phase I human clinical trials. Soon after, other principles of clinical pharmacology [e.g., pharmacokinetic-pharmacodynamic (PD) relationships] become critical to the evaluation and selection of the most appropriate dosing regimen of the drug in a carefully selected target population enrolled in phase II clinical trials. These trials form the scientific rationale for subsequent dose selection in large-scale phase III clinical trials where the primary goal is to provide adequate evidence of efficacy and relative safety of the drug. Phase III trials are the most expensive and time-consuming component of the overall drug development process and many believe that paying careful attention to doing clinical pharmacology "homework" has

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the greatest potential to reduce the failure rate of new drugs at this near-final stage of development.

Often, in parallel with phase III clinical trials, a group of clinical pharmacology studies, such as those in special populations, are conducted in human volunteers to develop a knowledge database of factors influencing drug exposure. These data are crucial for an understanding of when, and how much, to adjust dosage regimens. Because these studies typically focus on changes in systemic exposure, as a surrogate marker for either efficacy or toxicity, the availability and the intelligent use of exposure (e.g., dose, PK measurements)-response (e.g., biomarkers, surrogate clinical endpoints, clinical outcomes, PD) relationships to interpret the results of these studies become critical to information for various sections of the product label. These studies can be broadly classified into two broad categories: (1) those dealing with patient-intrinsic factors that include gender, age, race, diseases states (primarily renal and/or hepatic impairment), and genetic (e.g., activity of cytochrome P450 enzymes) factors, and (2) those dealing with patient-extrinsic factors that include drug-, herbal- and nutrient-drug interactions, environmental variables (e.g., smoking, diet), and lifestyle factors.

ROLE OF BIOPHARMACEUTICS

Related to the science of clinical pharmacology, biopharmaceutics can be thought of as the body of scientific principles applied to convert a well-characterized drug substance to an appropriate, and potentially optimized, drug product. At the heart of biopharmaceutics is a thorough understanding of the physical, chemical and biological properties of the drug substance related to absorption (e.g., solubility, stability and intestinal permeability) and how to utilize these data to decide on the best route of administration and to develop a successful dosage form. The development of an initial formulation for a drug substance entails the study of drug product dissolution under a variety of environmental conditions (e.g., pH), and linking the resulting rate and extent of dissolution to the subsequent rate and extent of absorption (i.e., bioavailability or BA). These so-called in vitro-in vivo correlations (IVIVC) are important to early optimization of formulation performance in order to achieve systemic plasma drug concentration-time profiles later in human clinical trials with the greatest chance for therapeutic success.

Not infrequently, the final, to-be-marketed formulation of the active drug substance is different than the initial formulations used in either early or late clinical trial phases of development. Biopharmaceutics plays a critical role in linking the in vivo performance or BA of each of the early formulations (i.e., reference formulations) to the final (i.e., test formulations) formulations.

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The standard study to assess comparative BA of the test and reference formulations is the bioequivalence (BE) study. Often, the results of BE studies are expressed as measures of exposure, such as area under the plasma concentration-time curve (AUC) and peak or maximum plasma concentration (Cmax). The ratio of these in vivo measurements (test/reference) are usually statistically reported as 90% confidence intervals (CI). BE is declared if the 90% CI is between 80 and 125% ("goalposts"). However, if the 90% CI is either partially or completely outside these "goalposts", therapeutic equivalence is determined by integrating the clinical pharmacology information about exposure-response relationships into the regulatory decision-making process.

REGULATORY REVIEW

Within the Center for Drug Evaluation and Research (CDER) of the FDA, the regulatory review of clinical pharmacology and biopharmaceutics studies is the responsibility of the Office of Clinical Pharmacology and Biopharmaceutics (OCPB). The mission of OCPB has patient care and therapeutics as center stage, and this is reflected by the scientific goals of clinical pharmacology and biopharmaceutics, that is, to critically study, thoroughly understand, and successfully identify (1) the right dose, in (2) the right dosage form, for (3) the right patient. The final step is to responsibly translate this knowledge to the product label with appropriate information about the use of the drug/drug product in the clinical pharmacology, precautions, warnings, contraindications, and/or dosage and administration sections of the package insert. This is indeed a critical step in the review process, since labeling a drug for use in the manner that is intended for patients to use it (or not use it) is one of the most important ways of risk management for ADRs.

OCPB's review process is based on a paradigm known as the Question-Based Review, or QBR [10]. It recognizes that it would be unreasonable to expect that everything will be known about the clinical pharmacology (CP) and biopharmaceutics (BP) of a drug/drug product at the time of NDA submission. Accordingly, the QBR emphasizes the importance of the reviewer's responsibility to ask the right questions related to the efficacy and safety of new medicines based on the clinical pharmacology and biopharmaceutics database provided by the sponsor in a NDA, and also to identify what is important but not known about the drug. The latter may be the basis for postmarketing studies (phase IV commitments). There are many critical principles in applying the QBR but two stick out the most when reviewing CP and BP studies: (1) analyzing study results and integrating knowledge thoughtfully across studies, and not just reviewing

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studies in isolation from one another, or necessarily in the chronological order in which they were conducted, and (2) interpreting results of CP and BP studies in the overall context of what is also known from the nonclinical chemistry, pharmacology and toxicology data, and the clinical efficacy and safety information, and not just to focus on providing a narrow-focused CP/BP report to medical officers. To meet these responsibilities, reviewers are strongly encouraged to act credibly and to communicate extensively with other professionals during the review process.

VIEW TOWARD THE FUTURE

Clinical pharmacologists and biopharmaceutical scientists have an opportunity, as much as any professional, to lead the pharmaceutical industry and regulatory agencies in leveraging their science and technology for achieving future breakthroughs in therapeutics. The process of marrying comprehensive biopharmaceutical information to clinical pharmacology data, and integrating that knowledge into what is known about drug efficacy and safety, will bring the drug development enterprise a step closer to realizing the dream of individualized medicine. Part of this process will be leveraging several existing fundamental technologies and new scientific discoveries to a greater extent.

Pharmacogenetics (PGt) and Pharmacogenomics (PGx)

While no consensus on definitions is at hand, for the purpose of this chapter PGt can be thought of as the study of the genetic variability in PK among individuals, affecting liver enzymes that metabolize drugs and transporters that determine BA and drug distribution. PGx, closely related to PGt, may be defined as the study of genetic variability, including that of drug receptors (PD), among individuals, affecting the rest of the genome that regulates drug response. Many believe that PGt and PGx are at the core of future drug development processes with applications ranging from new knowledge about the molecular basis of diseases to identification of new genes or gene products (e.g., protein) that serve as novel drug targets. There are several significant industry examples of the impact of PGt and PGx. These include (1) the comarketing of trastuzumab (Herceptin, Genentech) and a diagnostic test (HercepTest) for patients with breast cancer whose tumors have overexpressed HER 2 activity [11], (2) a gene-based diagnostic marker that has the potential to identify at-risk patients with HIV for hypersensitivity to abacavir (Ziagen, GSK), (3) haplotypes that have the potential to be used as diagnostic tests to optimize the selection of approved HMG Co-A reductase inhibitors ("statins") in patients with

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hypercholesteremia, and (4) potential genetic markers to identify patients with rheumatoid arthritis who are responders to IL-1 and TNF-inhibitors. A regulatory perspective on PGt and PGx has recently been published and regulatory agencies worldwide generally are optimistic that these sciences will, in time, profoundly transform the drug development and regulatory review processes [12].

However, closer attention needs to be paid to what is already known about PGt with an eye toward how this information can be integrated into current standards of patient care to reduce the incidence of ADRs. For example, it has been reported that of the top 27 drugs frequently cited in ADR reports, 59% are metabolized by at least one enzyme having poor metabolizer (PM) genotype. Eleven of the 27 drugs (38%), mainly used for cardiovascular and CNS diseases, are metabolized specifically by cytochrome P450 (CYP) 2D6 [13]. Despite the strong suggestion that knowing a patient's CYP 2D6 genotype (or phenotype), and adjusting doses downwards or upwards depending on the genotype, would positively influence benefit/risk of therapy, CYP 2D6 genotyping is not recommended in any package insert of approved products. There are a variety of reasons for this, but as genotyping tests for CYP enzyme activity become more widely available and cost-effective, clinical pharmacologists will have the responsibility to ask the right questions about genetic polymorphism and to act responsibly on the information during drug development and regulatory review.

In the broad world of PGx, there will be greater reliance on global DNA sequencing and candidate gene studies to discover genes and genetic biomarkers that play a role in assessing disease progression and variability in drug response. Clinical pharmacologists will have opportunities to explore associations between gene variants, in the form of single nucleotide polymorphisms (SNPs) or combinations of SNPs (haplotypes), to better understand variability in drug response and dosage requirements. In addition, complementary PGx technologies, such as gene-chip microarrays and quantitative polymerase chain reaction (PCR), will provide additional insights into the genetic basis of disease and drug response which will impact clinical therapeutics in terms of measuring disease- and drug-induced differences in expression profiles and providing multiple biomarker panels to associate with drug therapy.

Assay Development

It is well known that chemical assays of high quality (i.e., adequate sensitivity, selectivity, and reproducibility) are essential to obtaining credible data in clinical pharmacology studies (e.g., PK) and biopharmaceutics studies (e.g., BE). However, in the future, assay development that includes more sophisticated technologies and more attention to detail will be needed.

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For example, there are many pharmacological or physiological biomarkers of drug activity which are used in analyzing exposure-response relationships for the purpose of making decisions in drug development or regulatory review, where evidence of validation of the measurement of the response component is incomplete or missing. In addition, with the evolution of PGt and PGx, principles of validation of new technologies such as mass spectrometry (proteomics), high-throughput DNA sequencing, and expression profiling (microarrays) will need to be established to ensure credible interpretation and use of these data. Each of these newer technologies, in contrast to traditional technologies, will provide a tremendous amount of information about changes in gene expression and potentially useful biomarker panels. The bioinformatics software used to mine these data sets is not standardized at the moment, and as a result various association algorithms, cluster analyses, and SNP and haplotype identification methods are used from company to company. The potential for interlaboratory differences in interpretation is enormous and consensus on how to use these tools reliably will be important in clinical pharmacology and biopharmaceutics studies of the future.

Modeling and Clinical Trial Simulation (CTS)

Development and validation of models for exposure-response datasets have been widely used by clinical pharmacologists during drug development and regulatory review to understand the nature of dose-response and PK-PD relationships and to predict alternative clinical scenarios. There are many examples of the value of modeling in terms of improving drug development and regulatory review [14]. In the future, modeling of biological systems at the cellular level, disease progression models, and models for quantitative assessment of risk will take on greater importance in CP studies. More recently, CTS or computer assisted trial design (CATD) methodologies have been advanced as tools to use phase I and phase II exposure-response information to design phase III trials, predict trial outcomes in terms of efficacy and safety, and allow for more informed decisions on benefit/risk analysis and the economics of drug development programs [15]. CATD, while not routinely used in drug development and regulatory review, is likely to take on more importance as our understanding of the causes of disease, disease progression, molecular drug targets, and drug pharmacology/toxicology increases through the co-evolution of genetics and genomics.

Diagnostic Tests and Kits

As PGt and PGx mature, it is highly likely that gene-based diagnostic tests and kits using genetic markers will significantly influence drug

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development and regulatory review. These tests and kits will not only be used on patient blood or tissue samples to diagnose diseases when they are present, but will also be able to (1) predict the probability of developing-diseases in the future, (2) identify patients who are most likely to be responders or nonresponders, (3) select the most appropriate dose for a given individual, and (4) select the best drug in a class once a decision is made to institute drug therapy. To date, there are relatively few diagnostic test kits approved by FDA, although in the future this would be desirable. HercepTest (Dako Corporation) and PathVysion Her-2 DNA FISH (Vysis) have been approved by FDA to measure HER 2 activity prior to making a medical decision to administer Herceptin to women in advanced stages of breast cancer, and HIV-1 TruGene Assay (Applied Sciences/Visible Genetics) has been approved to measure HIV resistance and to provide drug treatment options for patients with AIDS. FDA approval of gene-based diagnostics would provide many advantages such as assuring high quality reagents, validated reference standards, standardized assay procedures and protocols, and greater acceptance of these tests by patients and physicians. Interpreting the test results for physicians, by bridging this information to package inserts, is likely to become an important responsibility of clinical pharmacologists in the future.

Knowledge Management (KM)

For the purposes of this chapter, KM is defined as the marriage of science, bioinformatics, and computer technology to more effectively assess and utilize the ever increasing amounts of clinical pharmacology and biopharmaceutics data arising from drug development. As an example, modern NDAs may contain more than 60 CP and BP studies, and each study contains many more pieces of data than ever before. In order to conduct a meaningful and thorough analyses of these data and to learn as much as possible about the drug/drug product, industry and regulatory scientists will need the capability that computer visualization and analysis software can offer. Applying web-based data management will enable endusers to (1) use information across studies better, (2) make more efficient and informed decisions about benefit/risk, and (3) create learning databases that can be effectively queried to compare CP and BP attributes across drugs and therapeutic areas. Visualization software is also a powerful way to communicate important CP and BP information to those in other disciplines in order to make maximum use of the scientific data at hand.

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SUMMARY

The current mission and goals of clinical pharmacology and biopharmaceutics is highly likely to expand and be transformed in the future as the new tools, technologies, and expectations (as described above and in the following chapters) become reality. Many of the questions about efficacy, safety, benefit/risk, drug dosing, and drug product performance will be tailor made for the scientists in CP and BP. These scientists will have to integrate their knowledge with other disciplines more broadly to take a leading role in drug development and regulatory decision-making. The efforts of clinical pharmacologists and biopharmaceuticists, if future challenges are accepted by the profession, will have the potential to introduce innovation and ultimately impact the standards of medical care. How CP and BP data is interpreted and applied in the future will affect risk assessment, risk management plans, and drug development and regulatory decisions. The quality of CP information in drug product labels and the setting of standards and specifications based on BP data to assure consistent drug product performance over time in the marketplace will likely impact the effectiveness and, perhaps most importantly, the safety of new medicines. This is, without a doubt, a common and meritorious goal shared by clinical pharmacologists and biopharmaceuticists whether they practise in industry or in regulatory agencies.

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2

Evolution of Drug Development and its Regulatory Process

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The history of clinical pharmacology over the past 100 years may be thought of as a gradual progression from the use of potions and other sometimes dubious concoctions to the complex drug development process seen today [1]. The future of clinical pharmacology has been described as academia, industry, and government working together to advance science, develop new drugs, and improve the quality of life of mankind [2]. Efforts such as the International Conference on Harmonization (ICH) have promoted unification of regulatory policies, including those related to clinical pharmacology. More than 35 harmonized ICH Guidelines are available [3] and the recently harmonized Common Technical Document provides for a common format for new drug and biological regulatory submissions. Following are perspectives from Europe and the United States on the progress of clinical pharmacology over the years, in these two major regions of the world.

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DRUG DEVELOPMENT IN EUROPE

Early Days

Clinical pharmacology, the science of drug actions in humans, started its development in the 19th century. Test animals were increasingly used in pharmacology research. In France, Francois Magendie (1783–1855) played a prominent role. He is known to many for his description of the foramen of Magendie in the brain but could be thought of also as one of the most important founders of modern pharmacology. Czech Jan Evangelista Purkinje (1787–1869), whose name is linked to large nerve cells in the brain (Purkinje cells) and to conducting tissue in the heart (Purkinje fibers), was one of the first to study drugs in healthy subjects, an unusual step, to avoid interference by illnesses when studying drug characteristics [4]. In 1805, German pharmacist Friedrich Serturner isolated the pure active ingredient in opium. He named this chemical morphine, after Morpheus, the Greek god of dreams. Serturner's discovery was the first isolation of an active ingredient. For many years he experimented on himself and others to explore the effects of the alkaloid.

In the 17th century, a controlled study design was described. Jan Baptista van Hellemont (1578–1644), a physician in Brussels, had proposed to his opponents to settle a dispute about wound treatments. Several hundred patients were to participate in an experiment, with vitriol or bloodletting treatments assigned by lottery to each individual patient. Results were to be judged by "the number of funerals" on each side. It is only in the 20th century that the randomized controlled study design became generally accepted. The double blind randomized study conducted in the late 1940s by the British Medical Research Council confirming the effect of streptomycin on tuberculosis was to become a classical example. With the emergence of the chemical industry in the second half of the 19th century, drug manufacturing by chemical synthesis became possible and a number of pharmaceutical companies emerged.

Several drugs to treat serious diseases were discovered. Due to insufficient pharmacological knowledge those drugs were probably too easily introduced. The American government realized an important role to play. Legislation in 1938 and later in 1962 required manufacturers to show respectively safety and efficacy of drugs. The American example was followed in Europe with some delay. In the Netherlands the first such legislation was introduced in 1958. But it was only after the thalidomide tragedy in the 1960s that an official agency to evaluate drugs started to operate efficiently in this country. Similarly, in the United Kingdom it was not until the Medicines Act was introduced in 1972 that evidence of efficacy as well as safety was required as a condition for granting a product license.

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The legal obligation to demonstrate safety and efficacy before market introduction stimulated the development of clinical pharmacology as a new scientific discipline. The development of clinical pharmacology is a logical consequence of the pharmaceutical revolution in the beginning of the 20th century and the increasing contribution that drug treatments have made to medical practice in the second half of the century [4, 5].

Clinical Pharmacology

Clinical pharmacology, the science of interactions between men and drugs, was forged as an established medical discipline in the late 1950s and early 1960s in the United States, the United Kingdom, and Scandinavia. By 1970, it had been recognized by World Health Organization (WHO) and in the same year the Clinical Pharmacology section of the British Pharmacological Society was formed. In 1974 the British Journal of Clinical Pharmacology was launched. Clinical pharmacology has developed unevenly within the European region and indeed throughout the world. It has developed rather at a faster pace in some countries (e.g., the United Kingdom, Scandinavia) but slower in others. The functions of clinical pharmacology were defined 30 years ago in a WHO report as research, teaching and service functions to enhance the "scientific study of drugs." Pharmacological service functions are referred to functions aiming to solve problems in drug therapy, not to traditional clinical work. In retrospect it is felt in Europe that most clinical pharmacology groups who lived up to the recommendation of this WHO report have evolved favorably, while many of those who did not, have disappeared [6].

There are different descriptions of clinical pharmacology. It is considered as both a research discipline (interdisciplinary) and a clinical specialty (specified training of MDs). Under ideal circumstances they work closely together, and there is a career ladder for both. At times, there has been tension between a conservative clinical specialist approach, at the cost of isolation, and a broader multidisciplinary-in-touch approach. However, to meet various challenges in Europe, old barriers divided along traditional subject lines, are being replaced in both academia and industry by interdisciplinary teams [6].

Four decades of clinical pharmacology research (1960–2000) have emphasized different aspects of the discipline (see Table 1) from controlled clinical trials and drug metabolism during the early 1960s to molecular pharmacogenetics and pharmacoeconomy during the late 1990s [6] (also see Section 2 of this chapter).

In Europe, clinical pharmacology continues to be driven by a thriving pharmaceutical industry, much of which is West-European based. Its

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TABLE 1 Four Decades and Different Aspects of Clinical Pharmacology [8]

- 1960–1970 Controlled clinical trial, adverse drug effects, drug metabolism, clinical pharmacokinetics.
- 1970–1980 Drug interactions, pharmacogenetics, therapeutic drug monitoring, improved methods to assess drug response, improved drug evaluation (Phases I and II).
- 1980–1990 Pharmacoepidemiology, pharmacovigilance, individualization of drug dosage scheduling, drug information.
- 1990–2000 Molecular pharmacogenetics, pharmacokinetic-pharmacodynamic modeling, population-based dose evaluation, pharmacokinetic optimization of drug effects, evidence-based pharmacotherapy, pharmacoeconomy.

development has been underpinned by the recognition that newly available drugs must be assessed in unbiased controlled clinical trials designed, conducted, and analyzed to the highest possible standards. Meanwhile, understanding of potential mechanisms of drug actions has improved, increasing the number of target sites for new drug development. Improved measurement techniques of both drugs and their metabolites, and the body's response to them, have increased the understanding of pharmacokinetics and pharmacodynamics [7].

Evolution in Clinical Drug Development

Globalization

Drug development is undertaken today mostly in a globalized industry where companies tap international sources of technology. European companies nurture U.S. as well as European scientific bases and vice versa. Traditional domestic companies are mostly less innovative and rather persist through marketing based strategies and protection [8]. Current trends in drug development are therefore global in nature. The items described in this section however reflect insights and opinions from European sources.

New Needs and Concepts

The implementation of genomic research combined with progress in discovery techniques has significantly increased the number of potential drug candidates for a series of diseases for which there are currently no or only insufficient treatments. Due to the present system, many of these candidates never reach the patient because of bottlenecks in, and limitations to, the drug development process (see Table 2). In the early 2000s, an

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TABLE 2 Bottlenecks in Traditional Drug Development [6]

- Insufficient research in the drug development process.
- Insufficient databases, benchmarking, and modeling tools for decision making.
- Lack of predictive models.
- Lack of integration of a range of technical and scientific disciplines.
- Insufficient data monitoring through the entire process.
- Insufficient industry-regulatory interface.
  Transparency and quality standards in the regulatory review process to be improved.

apparent downturn in productivity in pharmaceutical R&D has been observed. This is illustrated by the fact that the European Medicines Evaluation Agency (EMEA) has willingly given back part of its approved budget in 2002 because the anticipated number of new drug applications had not been forthcoming. European scientists from industry, academia, and drug regulators have been discussing the so-called "crisis." Many share the opinion that the rational way to reverse the trend of dwindling productivity is to introduce new faster methodologies and modern technology at every step of the development process [9–12].

To address new needs, a series of new concepts and techniques have been introduced in European drug development:

The need to predict the "developability" in the selection of potential drug candidates to go forward to full drug development. Early testing is expected to be discriminating while predictive of potential future problems, especially with respect to toxicity in humans [11].

The need to predict the probability of therapeutic and commercial success. Due to increasing costs of drug development and marketing competition, companies need an early answer to the likely clinical and commercial success with abandonment of the compound if the target profile is not likely to be met, ideally after the first human study [13]. In the end, economics are key considerations in drug development [14].

The increasing use of well-established techniques of PK modeling and the evaluation of dose-concentration-effect relationships (PK/PD) for both desired and undesired effects.

The use of rapidly evolving computer modeling and simulation techniques especially into difficult areas such as cancer and pediatric studies [11].

The need to optimize the dosing regimen early in clinical development. Traditional drug development, based on the "maximal tolerated dose"

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approach or fractions thereof, has often resulted in overdosing. However, clinical trials at too high a dose may attribute an unacceptable safety profile to an otherwise good drug [13]. Moreover, European regulatory authorities typically require an appropriate dose-finding study and demonstration of both the maximal tolerated and minimal effective dose.

Clinical development divided into two parts. "Exploratory" development or "proof-of-concept" which may require as little as one study and typically covers Phase I and Phase II (typically, Phase I studies conducted in healthy volunteers and Phase II in patient population) in the traditional theme, followed by "full" development and completion of the registration dossier. This approach is particularly important to innovative biotechnology companies which are considered of great value for the future. The probability of attracting a partner, and the value of partnership to the initial company, will depend heavily on whether the "proof-of-principle" point has been reached [13].

The use of well-validated surrogates which can substantially shorten clinical development time or time to reach a critical decision point. Biomarkers (less validated) may be useful in decision making, although a larger amount of data is usually required to offset the uncertainty. New biomarkers are explored in preclinical development and link preclinical pharmacology and toxicology with the design and interpretation of early human studies [13].

Pharmacogenetics gives researchers a powerful tool in the understanding of how genetic variation contributes to variations in response to medicines [15, 16]. Many individual and ethnic variations in drug metabolism have already been shown to be due to genetically determined variations in metabolic enzyme activity, particularly cytochrome P450 enzyme subtype polymorphisms. European regulators therefore require the testing of relevant drugs in target groups of poor or extensive metabolizers [17].

Integration of Knowledge

Projected needs of the pharmaceutical industry are related to the need for broad expertise to deal with increasingly complex projects and the integration of specialist knowledge. Optimization of the drug development process requires technical and scientific expertise in many areas. In some disciplines, such as genetics (human polyphormism), mathematics (modeling, simulation), bioinformatics (prediction), and information technology (including pharmacometrics and information management), there is a lack of well-trained experts. Moreover, due to the

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multidisciplinary nature of drug development, knowledge covering a range of disciplines is required [9].

An expected central challenge of the pharmaceutical industry in the coming years is the management of complex information. Many shortcomings in drug development can be attributed to insufficient use of available knowledge. The interfaces between the various phases of the R&D process have to be eliminated and a seamless discovery-development process established, ensuring that all knowledge and data are maintained and put to maximum use throughout (Fig. 1). New standards for handling complex data and standardization of the format for knowledge-exchange are required (A.Cohen, personal communication, 2001). This involves, developing IT-supported information data management and decisionmaking process [9]. For example, very promising new standards are to be used in view of the International Harmonization (ICH) initiatives, the Common Technical Document (CTD), and the Electronic Common

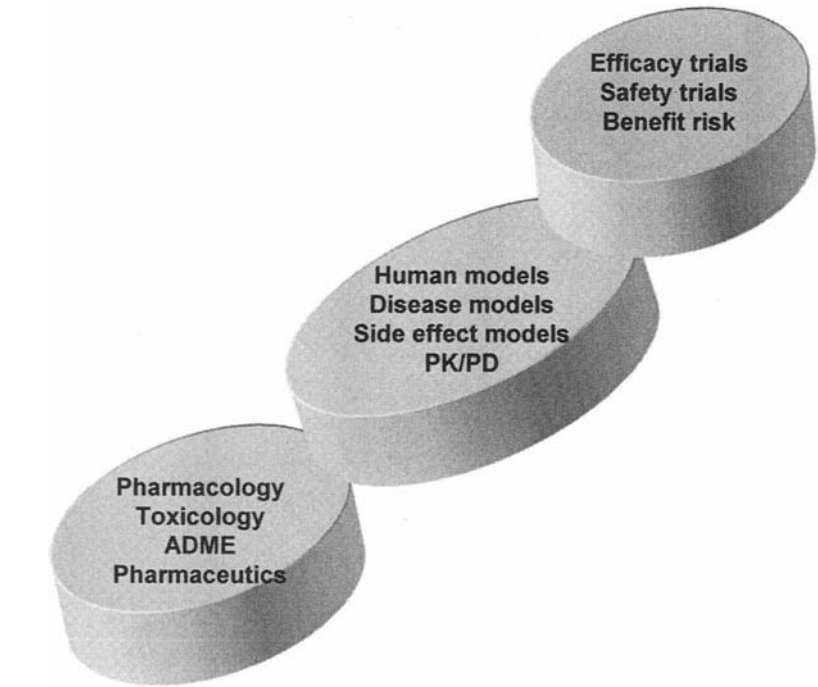

FIGURE 1 Integration of functions. Courtesy of A.Cohen, Center for Human Drug Research, Leiden, The Netherlands, Phase I studies tailored towards proof-of-concept. Personal communication, 2001.

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Technical Document (e-CTD). The aim is to provide a harmonized format and content for new product applications to be used with regulatory authorities in different regions of the world.

New Approaches in the Real World

The initial goals of drug evaluation have been modified to include new questions directed at goals other than drug safety and efficacy. For example, testing a drug in a population representing the "real" world setting has become a major basis for phase III trials and for establishing "evidence-based" pharmacotherapy.

Other new questions that have been asked are "How should the physician and patient be advised to use the drug?" and "Is the drug better or similar to a drug already available?" In a sense, clinical trials have evolved from a role in drug development to physician education and competitive marketing [18].

A frequently forgotten aspect of drug development, which in some respects is the most important of all, is defining the drug labeling, the European Summary of Product Characteristics (SmPC). This document should provide essential information for the health care professional and is the basis for patient instructions and prescribing guidelines. This document must be accurate but needs also to be easily understood [5].

Risk and Benefit

The standards of safety expected for an agent which may be lifesaving and one which relieves minor symptoms should not be the same. Perceptions on the appropriate balance of risk and benefit however vary widely, including nationally. Based on evidence of efficacy, which may be uncertain, together with limited safety data, licensing decisions may need to be made on as much a judgmental as a scientific basis [5]. While formal analysis of risk and benefit for a particular drug can be carried out, comparative risk assessment with similar drugs is also considered useful (see next paragraph).

Efficacy and safety have traditionally been the most important influential bases to make decisions. In the future, priorities may also be more influenced by costs and expected benefits of drugs on the market. At present pharmacoeconomic data are required for requesting reimbursement in countries such as Netherlands, United Kingdom, Denmark, Finland, Norway, and Portugal. In the future more information regarding the efficiency of the drug as compared to available drugs may be needed, thus magnifying the social value of the resources invested on drug expenditure [19].

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At the end, drug development should contribute to the use of the most appropriate drug to the right patient in an optimal dosage schedule with the right information and at a reasonable cost.

Considerations on Study Design

During the 1990s, the importance of properly designed early trials (Phase I and II) has led to dramatic changes in their design. These changes have included both proper randomized, double blinded designs and increased sample sizes. Although there are different opinions on how best to use data from Phase II in the present process, there is little doubt concerning the high level of information likely to be available at the end of Phase II and the conduct of too many Phase III and IV trials may be considered redundant or unethical [18].

There are global concerns that activities carried out during the later stages of clinical trials are balancing on the edge of inappropriate activities. Regulatory authorities in Europe have in a sense addressed these issues by their request, in specific situations, for comparative trials of marketed drugs. As the goal of these trials is often to show equivalence, they, however, tend to be more difficult to conduct and to require larger number of patients. Occasionally, global pharmaceutical companies have sought approval on the basis of placebo-controlled trials in the United States and have added active control comparative trials to register in Europe [18].

Problem Solving by the Entire Community

Mistakes in the design of a drug trial are usually reported as drug failure rather than insufficient expertise, marketing influence, inadequate regulatory management, or improper patient enrolment and follow up. The assumption has been made that these are problems for the pharmaceutical companies to solve. The regulatory role is simply to identify them and reject the failed studies. This might be considered false. It might be considered a problem created by the process of clinical trials, which should be solved by the entire healthcare community [18]. To address this and to reinforce the success of the European Agency, specific changes have been proposed to the European Commission to enlarge the scope of the Agency's activities beyond the evaluation of medicinal products, by strengthening its role as a scientific adviser.

"New Safe Medicines Faster" in Europe

Competitiveness of the Industry

Pharmaceutical companies based in Europe have traditionally played a leading role in developing new drugs, the industry making a significant

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TABLE 3 Objectives of New Safe Medicines Fast in Europe [7]

- The development of new technologies capable of more effective selection, development and approval of new, innovative and safe drugs.
- The use of such technologies to increase the capacity and speed of the pharmaceutical development process.
- The creation of a pan-European interdisciplinary network that bridges the gap between industry, academia, hospitals, and regulatory authorities.

contribution to the health and economy of European Union (EU) communities. Many of the top pharmaceutical companies reside in the EU and Switzerland and the European pharmaceutical industry has traditionally held a world-leading position. The trend in the late 1990s, however, indicated that U.S. companies have perhaps taken over the leadership role, showing the U.S. industry's superior ability to translate new technologies into marketable medicines [9].

However, initiatives to improve the EU competitive situation are the topic of agendas and programs of EU professional and trade organizations and a "New Safe Medicines Faster" initiative has been recognized for support by the European Commission [11]. Within Europe, medicinal development may still be hampered by barriers put up by the legislation of individual nations, by fragmentation and by suboptimal cooperation among the industry, academia, and authorities. The need for new revised European standards and for pan-European interdisciplinary networks is recognized and addressed [9].

Initiatives to Exploit Huge Opportunities

Proposed key actions are to promote basic research, new leading technologies, and new interface research, including management of the enormous quantity of diverse data that the development of drugs delivers. Networking is considered essential and the creation of centralized databases and database networks at a European level is suggested. New European platforms for regulators and researchers are recommended to design the necessary changes to the drug development process in partnership and bring about improvements in capacity, efficacy, and speed (Table 3). The purpose is to exploit the enormous opportunities created by the genomic revolution and modern drug discovery for the generation of new medicines to the benefit of the European citizen [9].

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The European System for Approving Medicines

Coordinating Scientific Resources

The role of national regulatory authorities in Europe has changed since the EMEA came into operation in 1995, after several years of cooperation among national authorities at a European level. The EMEA is a technical agency coordinating the scientific resources made available by the national authorities to provide high quality drug evaluations, to advise on development programs and to provide useful and clear information to the users. In addition to their country specific responsibility, national authorities now also investigate medicines for decisions at the EU level, in close collaboration with the drug regulatory authorities in other European countries [20].

To Promote Public Health and Free Circulation of Medicines

The European System offers two routes for granting authorizations. A company can or must, depending on the type of product, seek centralized approval, which means an authorization valid for the whole EU. The centralized procedure is compulsory for biotechnology products and optional for innovative conventional products. In this case the application is dealt with administratively by the EMEA. Independent evaluations are conducted by two selected members of the European scientific committee (named CPMP, Committee for Proprietary Medicinal Products). Multidisciplinary teams, coordinated by the selected members, perform those evaluations and discuss their conclusions with the other members. The European Commission makes final decisions after the CPMP has expressed an opinion following its scientific debate.

For innovative conventional products a company can instead choose the route based on mutual recognition of national decisions. The European System affords many advantages. New medicines come to market faster, which of course benefits patients and industry. Also, by utilizing the collective competence of several national drug authorities, the quality and objectivity of evaluations can be improved, duplication of work is avoided, and harmonized opinions and labeling throughout the EU becomes available.

An important part of this European-oriented work also revolves around developing new standards and requirements in the face of rapid scientific discoveries and development of new medicines. The intended end result is to promote public health and free circulation of medicines [20].

Broad Level of Satisfaction

In 2000, an extensive consultation [21] was carried out on behalf of the European Commission to review the operation of the new European System

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since 1995. It has revealed that there is a broad level of satisfaction about the system from ministries, patient and professional associations, regulatory authorities, and industry, although improvements can be made and new challenges exist.

There is a general feeling that the system has contributed to the creation of a harmonized EU market for medicinal products and that it provides a strong foundation for an efficient regulatory environment. There is also a general perception that assessment of products to date has provided a high degree of protection to the public health. This is despite the fact that there have been withdrawals from the market of products already authorized. This is considered consistent with increasingly effective pharmacovigilance procedures and the bias toward products developed on the leading edge of science.

Comparative Observations

From the same consultation in Europe, comparative observations upon the regulatory frameworks in the EU and United States have revealed a perception that the EU is taking a more risk-adverse approach to assessment as compared with the FDA's policy of risk management. Specific instances would exist where products were removed, or threatened with removal, from the EU market because of perceived safety concerns, while the same products were dealt within the United States by the imposition of specific warnings in the label [21]. Comments were made about a similar level of conservatism in the EU in the approach to the review of products in specialist areas such as oncology and a greater willingness to embrace new therapies in the United States [21].

Analysis of Outcomes

An analysis of outcomes of applications in the Central Procedure from 1995 to 1999, published by the EMEA [21], has shown 72% (97/135) positive outcomes, i.e., drug approvals. For applications with a negative outcome, methodological concerns over study design, choice of endpoint, comparator, and selected population were raised more frequently than over those with a positive outcome. FDA had authorized 13 (34%) of the 38 applications that had a negative outcome in the EU. This may be explained by a different attitude toward data requirements e.g., requirements for controlled data, by the availability to FDA of additional regulatory tools, e.g., conditional approvals, and by the limited use of EMEA scientific advice (11%) prior to submission [22].

It is expected that the Reform of EU Pharmaceutical Legislation, proposed in 2001, will influence the regulatory environment significantly [23].

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DRUG DEVELOPMENT IN THE UNITED STATES

The modern uses of clinical pharmacology data in the United States may be thought of as having several phases, beginning with early efforts in the 1970s, which related to the increased availability of sensitive and specific analytical methods around that time. This was followed by application of these capabilities to various areas such as the study of specific subpopulations. Further implementation has emphasized the link of pharmacokinetic data to clinical safety and efficacy data. Most recent emphasis has included better understanding of drug interactions and optimal dose adjustment for various sub-populations. Communication of information and recommended approaches has been facilitated by the preparation of FDA Guidances as well as ICH Guidelines.

Era of Pharmacokinetic Studies

The modern era of drug development related to clinical pharmacology studies may be thought to have begun in the 1970s. A key component was the development of bioanalytical methods needed to accurately detect plasma concentrations of administered drugs. This aspect has continued to improve until it is now possible to measure plasma levels for nearly every drug under development. This is an important factor in the study of the relationships of dose, exposure, and effect.

An important regulatory milestone was the creation of the distinct Human Pharmacokinetics and Bioavailability Section of NDAs [24]. This established a section in each NDA in which are contained all clinical pharmacology and biopharmaceutics studies. Prior to what is called the NDA rewrite, NDAs were not very consistent in content, and information to be included was not very precisely defined or well organized. When this Format and Content Guideline was first introduced in 1987, the types of studies were identified as:

- Pilot or background studies carried out in a small number of subjects as a preliminary assessment of ADME.
- BA/BE studies.
- Pharmacokinetic studies.
- Other in vivo studies such as those using pharmacological or clinical endpoints in humans or animals.
- In vitro studies such as dissolution and protein binding studies.

While the original focus was on in vivo studies in healthy subjects, this has expanded to include plasma sampling in patients as part of population pharmacokinetic studies, exposure response studies and pharmacokinetic/pharmacodynamic studies.

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There are numerous types of clinical pharmacology studies conducted during the development of a new drug. These include both studies on healthy subjects without the disease intended for treatment (Phase I) and studies involving patients (Phase II and III).

Studies in healthy subjects primarily focus on safety aspects of the drug, in establishing dose-toxicity relationships. These studies also investigate the pharmacokinetics for the drug under development, dose proportionality, absolute bioavailability, mass balance, effect of food, different formulations, as well as special populations.

Studies conducted in patients primarily relate to establishing efficacy and dose/response. In addition, optimal dosing interval, effect of severity of disease, tolerance, and adverse reactions are determined.

One significant example from this era involved a once-a-day extended release theophylline product which was shown to have a significant change in bioavailability when administered with a high fat meal. This important safety information resulted in the following precaution being added to the product's labeling:

> *Drug/Food Interactions* Taking (this product) less than one hour before a high-fat-content meal, such as 8 oz whole milk, 2 fried eggs, 2 bacon strips, 2 oz hashed brown potatoes, and 2 slices of buttered toast (about 985 calories, including approximately 71 g of fat) may result in a significant increase in peak serum level and in the extent of absorption of theophylline as compared to administration in the fasted state. In some cases (especially with doses of 900 mg or more taken less than one hour before a high-fat-content meal) serum theophylline levels may exceed the 20mcg/mL level, above which theophylline toxicity is more likely to occur.

A CDER Guidance [25] is available which describes current recommendations related to food effect studies and labeling based upon the results of such studies. Drug administration relative to meals is sometimes of great importance. The labeling for atovaqone serves to illustrate a situation where drug must be taken with food for optimal efficacy:

> Failure to administer (atovaquone) with meals may result in lower plasma atovaquone concentrations and may limit response to therapy.

Era of Special Populations

With the ability to conduct pharmacokinetic studies well established, attention advanced to additional applications. One such area was the study of various sub-populations, including the elderly, males compared to

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females and possible racial differences in pharmacokinetics. These aspects have continued to be emphasized and currently, it is expected that all NBAs will include analysis of data related to age, gender, and race.

CDER has used numerous methods to move forward the science of drug regulation. This includes involvement in Workshops to discuss current drug regulatory issues and the development of Guidances to put forward recommendations to sponsors as to how to proceed in many areas including clinical pharmacology studies. These Guidances include both CDER-developed documents [26] and ICH Guidelines [27].

The importance of age-related differences in response to drugs is discussed in a CDER Guidance [28]. A pharmacokinetic screen [29] is recommended, consisting of obtaining blood samples from patients in Phase II and Phase III clinical investigations. This is a means of identifying subgroups of patients, such as the elderly, in whom the drug may have unusual pharmacokinetic characteristics. Procedures such as the pharmacokinetic screen have evolved into current methods of population pharmacokinetics [30].

An example, from about 20 years ago, of a drug which proved to have serious toxicity among some elderly patients was benoxaprofen, a non-steroidal anti-inflammatory drug, used to treat arthritis. It was promoted as perhaps capable of "arresting the disease process" in rheumatoid arthritis. While it was certainly effective for labeled indications, for certain elderly patients it was associated with fatal cholestatic jaundice among other serious adverse reactions. If the pharmacokinetics of benoxaprofen had been studied in the elderly, it is possible that a dose adjustment for elderly could have been recommended and withdrawal of benoxaprofen from the market, which occurred in 1983, might have been avoided [31].

While for most drugs, males and females can safely receive the same dose, for a few drugs, differences in pharmacokinetics related to gender can be important. In 1993, the Guideline for the Study and Evaluation of Gender Differences in the Clinical Evaluation of Drugs [32] was published. This recommended inclusion of patients of both genders in drug development, assessment of clinical data by gender, assessment of potential pharmacokinetic differences between genders, and the conduct of specific additional studies in women, when appropriate.

Patients with impaired renal or hepatic function are also important subpopulations. Consideration of the need for dosage adjustment in situations of renal or hepatic impairment has received considerable attention. Guidances [33, 34] addressing these topics are available from FDA.

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Era of Drug Interactions and PK/PD Relationships

In 1991, a Workshop was held to discuss current thinking related to the rational integration of pharmacokinetics, pharmacodynamics, and toxicokinetics [35]. This was an important milestone along the path of closer relationships between clinical data and pharmacokinetic data.

In CDER, a reorganization establishing the Office of Clinical Pharmacology and Biopharmaceutics in conjunction with increased resources related to User Fees, promoted communication among medical reviewers and clinical pharmacology reviewers. Co-location of these reviewers provided for increased discussions, data sharing, and consultations.

The importance of the relationship of changes in pharmacokinetics to drug safety and efficacy is a continuing topic of much discussion. One related area is drug interactions, which sometimes are extremely important.

The interaction of fluorouracil and sorivudine, which caused a number of deaths in Japan [36] in the 1990s, served as an important reminder of the potential consequences of drug-drug interactions. Sorivudine was withdrawn in Japan after 15 patients who were prescribed both sorivudine and fluorouracil died. They had developed aplastic anemia, after taking sorivudine with fluorouracil. Knowing the situation that had occurred in Japan, sorivudine was not approved in the United States because of this potentially fatal drug interaction and the fact that alternative drugs to sorivudine were available, without the serious drug interaction potential.

Serious interactions between mibefridil and certain cholesterol lowering "statin" drugs resulted in the removal of mibefridil from the market. Mibefradil is a potent inhibitor of the metabolism of lovastatin and simvastatin and if either of these drugs is taken together with mibefridil, they can cause potentially life-threatening rhabdomyolysis related to much higher exposure to the statin drug due to inhibited metabolism caused by mibefridil [37].

In response to the significance of drug interactions, Guidances for the study of potential drug interactions, both in vitro [38] and in vivo [39], are available from FDA. Study continues on establishing in vitro/in vivo correlations for metabolically related drug interactions, in order to increase the predictability of in vitro drug interaction data.

An important new law went into effect in 1997. The Food and Drug Administration Modernization Act (FDAMA) [40] contained many new provisions including a section describing the number of required clinical investigations needed for approval. "If the Secretary determines, based on relevant science, that data from one adequate and well-controlled clinical

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investigation and confirmatory evidence (obtained prior to or after such investigation) are sufficient to establish effectiveness, the Secretary may consider such data and evidence to constitute substantial evidence…." The confirmatory evidence described can be obtained from earlier clinical trials, pharmacokinetic data, or other appropriate scientific data. This indicates further reliance on pharmacokinetic data in conjunction with clinical studies in the overall development of a new drug.

Year 2000 and Onward

As we continue to move forward in the area of clinical pharmacology aspects of drug development, we are faced with worldwide pharmaceutical companies, an explosion of data, and increased knowledge of the importance of optimal drug administration and the consequences of less than optimal drug use. In this context, computer-based systems increasingly provide an essential means of communication, as well as an effective tool for modeling and simulation. From the internet to personal information managers and Pocket PCs, we are nearly always close to a source of drug information. An increasingly common utterance is that there is so much information available but there are also increasing difficulties in sorting through this avalanche of information to find what is useful and thereby translating information into useful knowledge. But, there can be no question that computer-based information will continue to expand and progress as one of the most important means of communication and sources of information.

Clinical trial simulation [41] has matured to a point where all available information about a drug under development can be used efficiently to promote more rapid drug development. The entire process of drug development has been estimated to take up to 12 years and cost upwards of $350 million. About one-third of this cost and half the time is spent on clinical development. Simulation techniques can provide valuable information related to optimal dosing schedule, expected range of response, effects of changes in exclusion criteria on expected outcome, optimal frequency to measure response, and the impact of compliance.

Effective labeling has become an important topic, as large amounts of information become available for newly approved drugs. Drug interactions studied for a new drug have implications for the other drugs involved in the interactions and keeping labeling up to date for all drugs is a difficult task. As difficult is the task of healthcare providers being aware of all patient situations where dose adjustment may be appropriate, related to age, gender, race, renal or hepatic function, or drug interactions. FDA has proposed a new labeling format [42] in the effort to present important dosing and other safety information more clearly and obviously.

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The use of population pharmacokinetics [30] allows for the study of differences in safety and efficacy among population subgroups. This approach, which involves obtaining plasma samples from patients participating in clinical studies, can permit the identification of important factors, such as age, gender, weight, renal function, hepatic function, and concomitant medications which can affect the safe and effective use of a drug.

A topic of interest and considerable discussion recently is the Global Clinical Trial. Clinical trials conducted in the United States. Europe, or Japan often need some type of bridging study to allow the existing clinical data to be used in the approval process in a different region of the world. A Global Clinical Trial would include patients from the three ICH regions and might allow the results of the trial to be directly applicable for approval in all three regions and thereby speed worldwide drug approval.

Risk management is a frequently heard term in the current and future era of a complex healthcare environment, with many potent new drugs being approved, and an emerging global market. The FDAs Task Force on Risk Management [43] has recommended that a new framework for risk management activities is needed. The current system, which involves not only the FDA but also pharmaceutical manufacturers, healthcare practitioners, and patients, is more fragmented rather than part of an integrated systems effort. One important recommendation relates to risk confrontation, which involves community-based problem solving and involves all stakeholders in the decision-making process. Regarding post-marketing surveillance and risk assessment, it has been suggested that new approaches be considered such as increasing reliance on computer-based, perhaps global, health information databases, as well as gathering data from identified sentinel facilities where staff are trained to recognize rapidly, and report accurately, adverse reactions.

In conclusion, one of the most striking developments in this area over the past 30 years has been the change from independent clinical studies conducted in patients with the goal of determining safety and efficacy, and independent pharmacokinetic studies conducted in healthy subjects, to the current situation where these studies are viewed together. Over the years, these two sources of data have become increasingly associated and utilized together in numerous approaches to efficient drug development. By obtaining some additional plasma samples from patients in clinical studies, all studies in humans can be viewed as a continuum and a more complete evaluation of a drug can be obtained. By the integration of all available drug development data, dose can be better optimized for each patient, thereby minimizing adverse reactions and promoting effective treatment of diseases.

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3

Regulatory Bases for Clinical Pharmacology and Biopharmaceutics Information in a New Drug Application

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Within the United States, the development and marketing of products for human use in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or function of the body are regulated by legislation or law that has been enacted by the U.S. Congress. The responsibility to interpret, promulgate and enforce congressional legislation is given to the U.S. Food and Drug Administration (FDA) [1]. To assist in carrying out these responsibilities, the FDA implements rules or regulations that are published in the Federal Register (FR) then codified in the U.S. Code of Federal Regulations (CFR). Additionally, FDA publishes guidances that are not legally binding but are intended to provide insight and direction on how to best satisfy legislative and regulatory requirements plus they give the most current scientific thinking within FDA. In this chapter, key drug legislation, relevant CFR regulations, FDA guidances and more recent International Conference on Harmonization (ICH) guidelines that impact on, or are linked to, or provide input as to what clinical pharmacology and biopharmaceutics

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information should be provided in a new drug application (NDA) to support approval of a pharmaceutical product are reviewed. The parties involved in the ICH guidelines are regulatory authorities of Europe, Japan, and the United States, and experts from the pharmaceutical industry in the three regions.

The reader will notice, especially during the latter part of the chapter where individual guidances and guidelines are discussed, that there is quite a bit of overlap between the U.S. and the ICH documents as well as within the ICH documents. However, in the view of the authors, removing or minimizing this overlap would be a disservice to these documents and so even at the risk of being repetitious, regulatory basis which support clinical pharmacology and biopharmaceutic information from all the relevant documents is presented.

For the purpose of this chapter, clinical pharmacology is interpreted to encompass (i) that which the body does to a drug in terms of absorption, distribution, biotransformation and excretion (i.e., its pharmacokinetics (PK) and exposure characteristics) and (ii) what the drug and/or its metabolite(s) do to the body in terms of mechanism(s) of action and resultant biochemical, physiological, and/or clinical effects or outcomes (i.e., its pharmacodynamics (PD) or response characteristics) when administered to healthy subjects and/or the target patient population(s) that may include "special populations" where dose and/or dosing regimen changes may or may not be needed. Biopharmaceutics is interpreted to encompass the characterization of the physical and chemical properties of a drug and/or its dosage form(s) along with determining performance characteristics via in vitro and/or in vivo procedures or methodologies. Often clinical pharmacology and biopharmceutics information overlap.

U.S. DRUG LEGISLATION

In the U.S., the key piece of legislation or law that sets the framework to insure that safe and effective pharmaceutical products reach and are maintained in the marketplace is the Federal Food, Drug and Cosmetic Act (FDCA)[1] [http://www.fda.gov/opacom/laws/fdcact/fdctoc.htm] [1]. Today's version of the FDCA is the culmination of numerous modifications or amendments to the original legislation that was enacted in 1938 as the result of deaths due to a sulfanilamide product that contained diethylene glycol or antifreeze in the formulation. The 1938 FDCA set a requirement that safety needed to be demonstrated for drugs and before a new drug could be introduced into interstate commerce a new drug application (NDA) needed to be submitted to FDA. Drug products marketed before 1938 were however exempted from the FDCA (i.e., "grandfather drugs").

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Historical and more current amendments to the FDCA include the Durham-Humphrey Amendment of 1951, the Kefauver-Harris Amendments of 1962, the Drug Listing Act of 1972, the National Environmental Policy Act of 1974, Medical Device Amendments of 1976, the Orphan Drug Act of 1983, the Drug Price Competition, and Patent Term Restoration Act of 1984 (i.e., Waxman-Hatch Amendments), the Drug Exports Amendments Act of 1986, the Prescription Drug Marketing Act of 1988, the Safe Medical Devices Act of 1990, the Prescription Drug User Fee Act (PDUFA) of 1992, the FDA Modernization Act (FDAMA) of 1997 and the Best Pharmaceuticals Act for Children of 2002. Of the nine chapters in the present FDCA, the key chapters and sections related to drugs include and address the following.

Chapter II of FDCA—Definitions (Section 201)

In this section, definitions for key terms like drug, interstate commerce, labeling, etc. are given.

Chapter III of FDCA—Prohibited Acts and Penalties (Sections 301–310)

Identified in these sections are different actions or scenarios that are prohibited for drug products intended for interstate commerce (e.g., introduction of adulterated or misbranded products, etc.). Also identified are the legal consequences that can occur, which include criminal charges, monetary penalties and/or seizures if one is involved in actions or scenarios that are defined as prohibited.

Chapter V of FDCA—Drugs and Devices (Sections 501–563)

Sections 501 and 502—Adulterated and Misbranded Drugs

Within Chapter V, Section 501 addresses when a drug shall be deemed adulterated. It raises the fact that regulations can be promulgated to prescribe appropriate tests or methods of assay for the determination of strength, quality, or purity of drugs if such tests or methods are not set forth in an official compendium (i.e., the "United States Pharmacopoeia and the Homoeopathic Pharmacopoeia of the Unites States"). Section 502 addresses when a drug shall be deemed misbranded.

Section 505—New Drugs

Of the different chapters and sections covered in the FDCA, it is Section 505 of Chapter V for *New Drugs* which sets the overall foundation or basis for

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having pharmaceutical manufacturers or sponsors submit information to FDA before a product is allowed to market. Section 505 establishes that before the introduction of any new product into interstate commerce, an application needs to be filed with FDA for approval. Under Sections 505(b)(1), 505(b)(2), and 505(j), three types of drug applications are described. It is noted that Sections 505(b)(2) and 505(j) are the result of the Drug Price Competition and Patent Term Restoration Act of 1984. Together, these two sections replaced FDA's *paper NDA policy* that permitted an applicant to rely on studies published in the scientific literature to demonstrate safety and effectiveness of duplicates of certain post-1962[2] innovator or pioneer drug products.

For an NDA that is covered under 505(b)(1), the application contains full reports of clinical investigations of safety and effectiveness that are conducted by or for the applicant. For an NDA covered under 505(b)(2), one or more of the safety and effectiveness investigations used to support the application's approval are not conducted by or for the applicant and the applicant has not obtained a right of reference or use from the person by or for whom the investigations are conducted. Section 505(b)(2) allows for the approval of products other than generic products (see below) and it permits the use of literature or an Agency finding of safety and/or effectiveness of a FDA-approved drug to support the approval of a product.

In addition to safety and efficacy information. Section 505 also indicates that 505(b)(1) and (2) applications need to provide(i) a list of the articles used as components for the drug, (ii) a statement of the composition of the drug, (iii) a description of the methods used in, and the facilities and controls used for the manufacture, processing, and packing of the drug, (iv) samples of the drug and the articles used as components if requested, and (v) samples of the proposed labeling.

The third type of application is a 505(j) application that is also known as an abbreviated new drug application (ANDA). The 505(j) application is for duplicates of already approved products, or generic products, and although it is beyond the scope of this chapter, it is noted that such an application is to contain, among other things, information to show that the product for approval is the same in active ingredient, dosage form, strength, route of administration, labeling and performance characteristics (i.e., is bioequivalent) as that of a previously approved product (i.e., the reference listed drug or RLD), that is, unless a suitability petition is filed and accepted, for example, for a different active ingredient in a combination drug product, or a different dosage form, strength or route of administration than the RLD.

If a generic product is found to be bioequivalent to the RLD and it is approved, it will then be included in the FDA reference text entitled,

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Approved Drug Products with Therapeutic Equivalence Evaluations which is often referred to as the "Orange Book"[3] [http://www.fda.gov/cder/orange/default.htm] [2]. In this book, a generic product that is bioequivalent to the RLD will be assigned a code of "A" which means that it can be substituted for the RLD product or any other generic product that is approved and coded A.

Via Section 505(i), the bases for dealing with new pharmaceuticals that are under investigation or development prior to filing an NDA are addressed (i.e., investigational new drug (IND) applications). This section indicates that regulations should be promulgated to address the investigational situation for new drugs. It further indicates that a clinical investigation for a new drug may begin 30 days after the applicant has submitted information about the drug and the intended clinical investigation. The information to be provided should include a description of the design of the clinical investigation plus information to allow an assessment of safety that is to include "adequate information on the chemistry and manufacturing of the drug, controls available for the drug and primary data tabulations from animal studies or human studies." A clinical investigation may be prevented from being initiated during the 30-day window of time (i.e., a "clinical hold") if insufficient information is provided to allow for assessment of safety considerations, or there are real safety concerns based on the information that is provided. Following the initial IND clinical investigation, the FDA allows subsequent IND clinical investigations to not be restricted to the 30-day requirement before a study can be started. However, a clinical hold can be imposed on any IND investigation before it is started or after it is initiated if there are justified safety concerns.

Section 505A—Pediatric Studies of Drugs

As a result of the FDA Modernization Act (1997) [http://www.fda.gov/cder/fdama], the FDCA was amended to address pediatric drug studies among other things. If it was determined (i) for 505(b)(l) applications before a new drug's approval (i.e., before 2002), or (ii) for an already approved drug that is identified on a list prepared by FDA, that information related to the use of the drug in the pediatric population may provide health benefits to this population, a written request could be sent to the drug manufacturer or sponsor to conduct a pediatric study(s). Pediatric studies may only need to include "pharmacokinetic studies," if appropriate, as compared to the more classical clinical safety and efficacy studies. This assumes that (i) the disease being treated or diagnosed is similar in nature between adult and pediatric patients, (ii) there would be a similar safety profile between adult and pediatric patients, and (iii) there are similar PK (and PD relationships if known) between the two populations. If a study(s) is carried out as

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requested and specified by FDA, the applicant could obtain six months of additional marketing exclusivity for an NDA. After January 1, 2002 all newly submitted NDAs must include pediatric information if appropriate. However, the 2002 Best Pharmaceuticals Act for Children extended the time to allow drug sponsors to apply for six months marketing exclusivity until October 2007 for both new NDAs or drugs on FDAs list for which pediatric information would be important to obtain.

Section 506—Fast Track Products

To facilitate the development and to expedite the review of a drug product for the treatment of a serious or life-threatening condition where the product demonstrates the potential to address unmet medical needs for the condition, Section 506 addresses this situation. The fast track approval of such a product can be based on the determination that the product has an effect on a clinical endpoint or on a surrogate endpoint that is reasonably likely to predict clinical benefit. However, the approval of a fast track product may be subject to a requirement that the sponsor conduct appropriate postapproval studies to validate the surrogate endpoint or otherwise confirm the effect on the clinical endpoint within a specified time.

Section 506A—Manufacturing Changes

For manufacturing changes, they are addressed in Section 506A. This section discusses "major" and other manufacturing changes in a general sense and touches upon when a supplemental application to an NDA is needed to support a change. A manufacturing change is considered a major change if it is determined to have substantial potential to adversely affect the identity, strength, quality, purity, or potency of the drug as they may relate to the safety or effectiveness of the drug. Related criteria include (i) a qualitative or quantitative formulation change for the involved drug or a change in specifications in the approved application, (ii) the determination by regulation or guidance that completion of an appropriate clinical study demonstrating equivalence of the drug to the drug as manufactured without the change is required, or (iii) a change determined by regulation or guidance to have a substantial potential to adversely affect the safety or effectiveness of the drug.

Sections 525 to 528—Drugs for Rare Diseases or Conditions

These sections are the result of the Orphan Drug Act of 1983. The Pharmaceuticals that are covered are for diseases or conditions that are rare in the United States. A "rare disease or condition" is defined as any disease

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or condition that (i) affects less than 200,000 persons in the U.S. or (ii) affects more than 200,000 persons in the U.S. for which there is no reasonable expectation that the cost of developing and making the drug available will be recovered from U.S. sales. This section further explains that a manufacturer or sponsor needs to request that a drug be designated for a rare disease or condition before the submission of an application under Section 505(b).

For a drug that is given orphan drug status, the expectations are that similar clinical pharmacology and biopharmaceutics information would be provided in an NDA as that for a drug that is not given the orphan drug status.

Chapter VII of FDCA—Fees Relating to Drugs (Sections 735–736)

This chapter and its sections are the result of the Prescription Drug User Fee Act of 1992. Under this part of the FDCA, fees are authorized and specified as to what is to be charged to a drug manufacturer or sponsor who submits a human drug application via 505(b)(1) or 505(b)(2), or as a supplement to such an approved application. The fees are to cover the expenses that are incurred for the review of an application. As a result of a reauthorization in 1997, fees are now not to extend past October 1, 2002 unless there is another reauthorization.

CFR REGULATIONS

As has been previously covered, FDA is given the responsibility to interpret, promulgate and enforce U.S. drug legislation, or more specifically the FDCA. The FDCA, although being quite specific in some sections as to what the intent and expectations are, other sections allow for further clarification or interpretation of the intent, expectations and/or what is needed or required to comply with and enforce the law. As previously noted, to assist in carrying out its responsibilities related to the FDCA, FDA will publish notices, proposed rules, and regulations plus finalized rules and regulations in the FR [3] followed by codification of finalized rules and regulations in the CFR[4] [4]. For the purpose of this chapter, only highlights from parts 300.50, 312, 314, and 320 of Chapter I (Food and Drug Administration, Department of Health and Human Services) of Title 21 (Food and Drugs) of the CFR will be covered.

For the different CFR parts, when taking into account this chapter's objective of addressing the regulatory bases for needing clinical pharmacology and biopharmaceutics information in a NDA, they will be covered in a sequence and cross referenced as appropriate to allow for a

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more interrelated perspective as needed. For complete content of the discussed parts, readers are referred to the CFR.

21 CFR 320—Bioavailability and Bioequivalence Requirements

Historically, part 320 that addresses bioavailability (BA) and bioequivalence (BE) requirements was the outcome of a 1974 report that was prepared by the Drug Bioequivalence Study Panel that was convened under the U.S. Congress Office of Technology Assessment [5]. The charge to the panel was to "examine the relationships between chemical and therapeutic equivalence of drug products and to assess the capability of current technology—short of therapeutic trials in man—to determine whether drug products with the same physical and chemical composition produce comparable therapeutic effects." In the report one conclusion was that the standards and regulatory practices at the time did not insure bioequivalence for drug products. The report went on to make recommendations as to what could be done. As a result, in 1977 FDA finalized its Bioavailability and Bioequivalence Requirements via the FR which were subsequently codified in the CFR.

Although the impetus for the BA and BE requirements was for assuring therapeutic equivalence among duplicate or generic products, the requirements were also crafted to establish information needs to support the approval of NDAs for new molecular entities (NMEs) or new chemical entities (NCEs), as well as for defined changes for already approved NDA products. The inclusion of requirements for NDAs was to (i) foster better product quality, (ii) define or characterize what happens to a drug and its dosage form(s) when administered, (iii) provide information to help understand or interpret clinical safety and efficacy findings as appropriate, and (iv) provide useful information via the product's labeling or package insert for healthcare professionals.

Under Section 320.1, definitions are provided. The term bioavailability is defined as the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. It further states that for drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect that rate and extent to which the active ingredient or active moiety becomes available at the site of action. Other terms that are defined include bioequivalence, drug product, pharmaceutical equivalents, and pharmaceutical alternatives (see Glossary).

For part 320, key sections and subsections include the following, for which some are expanded upon as needed.

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- 320.21 Requirements for submission of in vivo bioavailability and bioequivalence data.

  Under this section, as related to NDAs, it indicates that "Any person submitting a full new application to the FDA shall include in the application either:

  1. Evidence demonstrating the in vivo bioavailability of the drug product that is the subject of the application; or
  2. Information to permit FDA to waive the submission of evidence demonstrating in vivo bioavailability."

  This section goes on to indicate that any person submitting a supplemental application to FDA shall include in the supplemental application evidence demonstrating the in vivo bioavailability of the product or information to permit FDA to waive the submission of evidence demonstrating in vivo bioavailability for changes that include:

  1. A change in the manufacturing process, including a change in product formulation or dosage strength, beyond the variations provided for in the approved application.
  2. A change in the labeling to provide for a new indication for use of the drug product, if clinical studies are required to support the new indication for use.
  3. A change in the labeling to provide for a new dosage regimen or for an additional dosage regimen for a special patient population, e.g., infants, if clinical studies are required to support the new or additional dosage regimen.

- 320.22 Criteria for waiver of evidence of in vivo bioavailability or bioequivalence.
- 320.23 Basis for demonstrating in vivo bioavailability or bioequivalence.
- 320.24 Types of evidence to establish bioavailability or bioequivalence.

  This section covers the different types of in vivo and in vitro methods that can be used to determine bioavailability and bioequivalence. They are ranked in descending order of accuracy, sensitivity and reproducibility as stated or summarized as follows:

  1. i. An in vivo test in humans in which the concentration of the active ingredient or active moiety, and, when appropriate, its active metabolite(s), in whole blood,

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plasma, serum, or other appropriate biological fluid is measured as a function of time,

ii. An in vitro test that has been correlated with and is predictive of human bioavailability data; or

iii. An in vivo test in animals that has been correlated with and is predictive of human bioavailability data.

2. An in vivo test in humans in which the urinary excretion of the active moiety, and, when appropriate, its active metabolite(s), are measured as a function of time.
3. An in vivo test in humans in which an appropriate acute pharmacological effect of the active moiety, and, when appropriate, its active metabolite(s), are measured as a function of time if such effect can be measured with sufficient accuracy, sensitivity, and reproducibility.
4. Well-controlled clinical trials in humans that establish the safety and effectiveness of the drug product, for purposes of establishing bioavailability, or appropriately designed comparative clinical trials, for purposes of establishing bioequivalence.
5. A currently available in vitro test acceptable to FDA (usually a dissolution rate test) that ensures human in vivo bioavailability.
6. Any other approach deemed adequate by FDA to establish bioavailability and bioequi valence.

- 320.25 Guidelines for the conduct of an in vivo bioavailability study.

Subheadings for the subsections under this section include:

a. Guiding principles.
b. Basic design.
c. Comparison to a reference material.
d. Previously unmarketed active drug ingredients or therapeutic moieties.
e. New formulations of active drug ingredients or therapeutic moieties approved for marketing.
f. Controlled release formulations.
g. Combination drug products.
h. Use of a placebo as the reference material.
i. Standards for test drug product and reference material.

Related to subsection (d) that addresses previously unmarketed active drug ingredients or therapeutic moieties, it states that the

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purpose of an in vivo bioavailability study is to determine the bioavailability of the formulation proposed for marketing as well as to determine essential pharmacokinetic characteristics of the active drug ingredient or therapeutic moiety *such as* rate of absorption, extent of absorption, half-life, excretion, metabolism, and dose proportionality. It further indicates that such characterization is a necessary part of the investigation of the drug to support drug labeling.

Under the umbrella to support drug labeling as outlined in this subsection, and with the experience that has been obtained over time since implementation of the BA and BE Requirements, along with advances in technology, updated and added information needs, in the realm of clinical pharmacology and biopharmaceutics (as defined above and under the purview of 21 CFR 320), are being asked to be addressed by sponsors in their drug development programs for new products. As will be covered in the section that discusses FDA guidances, FDA provides more current thinking on such information needs as related to the different aspects of clinical pharmacology and biopharmaceutics. (Note: Likewise in ICH guidelines, they too present and expand upon information needs in the areas of clinical pharmacology and biopharmaceutics for drug product registration, most of which is consistent with FDA guidances.)

- 320.26 Guidelines on the design of a single-dose in vivo bioavailability study.
- 320.27 Guidelines on the design of a multiple-dose in vivo bioavailability study.

21 CFR 300.50—Combination Drugs

Under this CFR part it addresses fixed-combination prescription drugs for humans. It states that "Two or more drugs may be combined in a single dosage form when each component makes a contribution to the claimed effects and the dosage of each component (amount, frequency, duration) is such that the combination is safe and effective for a significant patient population requiring such concurrent therapy as defined in the labeling for the drug." It further explains that special cases of this general rule are where a component is added (i) to enhance the safety or effectiveness of the principal active component and (ii) to minimize the potential for abuse of the principal active ingredient.

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Related to 21 CFR 300.50 from a clinical pharmacology and biopharmaceutics perspective, specifically for the scenario where the new combination product is to be administered as an alternative to giving two or more currently marketed, single ingredient products, one is referred to 21 CFR 320.25 (g) as identified above. Here it indicates that an in vivo bioavailability study is needed to determine if the rate and extent of absorption of each active drug ingredient or therapeutic moiety of the combination product is equivalent to the rate and extent of absorption of each active drug ingredient or therapeutic moiety administered concurrently in separate single-ingredient preparations. Information to address drug-drug interaction implications for the two or more drugs in a combination product is also usually needed.

21 CFR 312—Investigational New Drug Application

Within 21 CFR 312, some of what is presented is addressed in Section 505(i) of Chapter V of the FDCA as covered above. However, within 21 CFR 312 expanded and more detailed information related to INDs is given (e.g., information related to IND content and format, type of IND amendments and reports, administrative related actions, responsibilities of sponsors and investigators, etc.).

Of note, under Section 312.21, it indicates that the clinical investigation of a previously untested drug is generally divided into three phases (Phases 1, 2, and 3). In general the phases are carried out sequentially but they may overlap.

Phase 1 is where the initial introduction of an investigational new drug into humans occurs. The studies in Phase 1 are designed to determine the metabolism and pharmacologic actions of the drug, side effects associated with increasing doses and, if possible, obtain early evidence of effectiveness. Ideally, sufficient information about the drug's pharmacokinetics and systemic exposure plus pharmacological effects or pharmacodynamics should be obtained to permit the design of well-controlled, scientifically sound Phase 2 studies. The number of subjects or patients used in Phase 1 studies can vary with the drug but is usually in the range of 20–80.

Phase 2 is where well-controlled clinical studies are conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study. Also determined are the short-term side effects or risks associated with the drug or product. The number of patients used in Phase 2 studies is usually no more than several hundred.

Phase 3 studies include controlled and uncontrolled trials that are intended to gather additional information about effectiveness and safety for

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evaluating the drug's overall benefit-risk relationship and to provide adequate information for labeling. Phase 3 studies can include from several hundred to thousands of patients.

Ultimately when an NDA is submitted to FDA, it includes all of the studies that have been carried out in Phases 1, 2, and 3. Human clinical pharmacology and biopharmaceutics information is most often obtained from studies that are conducted as Phase 1 type studies, but with the advent of important and useful ways to analyze and model PK and PD data, including population PK and PD statistical approaches, information can and is being obtained in Phase 2 and 3 studies. There are FDA and ICH guidances and guidelines summarized below, which give insight into this.

Lastly, in Section 312.85 there is discussion on Phase 4 studies. At the time FDA is considering giving an NDA approval it may, with concurrence from the NDA sponsor, request that an additional postmarketing study or studies be conducted to delineate additional information about the drug's risks, benefits, and optimal use. Phase 4 type studies can be and are requested to obtain additional clinical pharmacology- or biopharmaceuticsrelated information if warranted.

21 CFR 314—Applications for FDA Approval to Market a New Drug or an Antibiotic Drug

Like for 21 CFR 312, some of what is covered in 21 CFR 314 as related to applications for market approval for a new drug is also covered in Sections 505(b) and (j) of Chapter V of the FDCA. However, 21 CFR 314 is much more expansive and specific in addressing NDAs (and AND As) as to the procedures and requirements for the submission to, and for the review by FDA of such applications for approval. Also addressed are amendments, supplements, and postmarketing reports to applications.

Under 314.2 it states that the purpose of 21 CFR 314 is to establish an efficient and thorough drug review process in order to (i) facilitate the approval of drugs shown to be safe and effective and (ii) ensure the disapproval of drugs not shown to be safe and effective. Additionally, it addresses the establishment of a system for FDAs surveillance of marketed drugs. Via Section 314.50 it covers the content and format of an NDA application that is to include summary sections and technical sections for the areas of (i) chemistry, manufacturing, and controls, (ii) nonclinical pharmacology and toxicology, (iii) human pharmacokinetics and bioavailability, (iv) microbiology, and (v) clinical data along with statistical analyses.

For clinical pharmacology and biopharmaceutics related information, 314.50(d)(3) indicates that a technical section should include human

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pharmacokinetic data and human bioavailability data, or information supporting a waiver of the submission of in vivo bioavailability data as covered under 21 CFR 320. Further it indicates that a description of each of the human pharmacokinetic and bioavailability studies performed by or on behalf of the applicant should be provided along with a description of the analytical and statistical methods used plus a statement related to informed consent procedures used per study. Additionally, if the application describes—in the chemistry, manufacturing, and controls sections—specifications or analytical methods needed to assure the bioavailability of the drug product or drug substance, or both, a statement of the rationale for establishing the specifications or analytical methods, including data and information supporting the rationale should be provided. Lastly, it is indicated that there should be summarizing discussion and analysis of the pharmacokinetics and metabolism of the active ingredients and the bioavailability or bioequivalence, or both, of the drug product. In addition to what is covered in 21 CFR 314, 21 CFR 320 plus FDA guidances and ICH guidelines should additionally be consulted to get further insight as to what specific clinical pharmacology and biopharmaceutics information and data should be provided in an NDA.

FDA GUIDANCES

Like the FR and CFR that are often used to better clarify or define the intent, expectations, or what is needed or required to comply with or enforce the FDCA, FDA, as already noted, prepares and publishes guidances that provide further insight, direction, and the Agency's current thinking on how to best satisfy the FDCA and FR/CFR rules or regulations, albeit that FDA guidances are not legally binding. FDA guidances also attempt to establish uniformity and consistency as to what is needed in NDAs for submission.[5] Key FDA guidances [http://www.fda.gov/cder/guidance] that address different aspects of clinical pharmacology and biopharmaceutics, as previously defined, are covered.

Please note that only the guidances that are posted as "final" on the CDER web page are summarized below and the reader is encouraged to look up guidances that are posted but are at the "draft" stage. Additionally, several of these "final" guidances deal with either a particular drug product or a specific therapeutic area and therefore are not considered in this chapter; only the "final" guidances that cover the general, broad-based principles which apply to majority of the drug products and therapeutic areas are summarized below.

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Clinical Pharmacology

"Format and Content of the Human Pharmacokinetics and Bioavailability Section of an Application" Guidance (1997)

This guidance is actually a reissuance of the guideline with the same title that was issued in 1987 and is intended to assist applicants to prepare the Human Pharmacokinetics and Bioavailability section of an NDA. After providing a brief overview of what types of studies are generally expected for NDAs, the guidance provides the outline of format for this section. The section should contain, in a tabular presentation, a summary of the studies, data, and overall conclusions, drug formulation, analytical methods, and a product in vitro release method (e.g., dissolution) if appropriate. The tabular format, with columns identifying specific variables for each of these components, is provided in the appendix. Finally, individual study report format and other considerations are covered. It should be noted that even though the guideline was created almost 15 years ago, this is an excellent document and the formatting recommendations conveyed here are followed, as a minimum, to date by most applicants.

For last several years, there has been a lot of activity and extremely thoughtful efforts at the ICH level and a recently issued ICH guideline called the common technical document (CTD) provides an expanded and updated version of this guideline. This and other relevant ICH documents are covered later in the chapter.

"Guideline for the Study of Drugs Likely to be used in the Elderly" (1989)

Even though written 12 years ago with the primary intent to advice sponsors on how to undertake clinical investigation of drugs likely to be used in the elderly, this guideline is a milestone in terms of identifying, explaining, and recommending clinical pharmacology studies in terms of drug-drug interactions, drug-disease interactions, special populations (elderly, renally impaired and hepatically impaired), and pharmacodynamic studies (in the elderly). Further, this guideline also established the concept of "Pharmacokinetic Screen" which has subsequently matured into the science of "Population Pharmacokinetics." In view of the authors, this is a must-read classical document. Not surprisingly, this is also one of the first topics that were finalized at the ICH and in view of the authors, the E7 document, namely "Clinical Trials in Special Populations—Geriatrics" is an excellent update of this '89 document. The E7 document is covered in detail later on in the chapter.

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"Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies In Vitro" Guidance (1998)

This guidance is directed towards a broad class of drugs, namely molecules with a molecular weight below 10 kilo Daltons, and it provides suggestions on current approaches to in vitro studies of metabolism and interactions of such molecules. The guidance is intended to encourage routine, thorough evaluation of metabolism and interactions in vitro whenever feasible and appropriate. This guidance recognizes that the importance of such an approach will vary depending on the drug in development and its intended clinical use. It also recognizes that clinical observations can address some of the same issues identified in this document as being susceptible to in vitro study.

The guidance covers the following topics: observations and conclusions; techniques and approaches for in vitro studies for drug metabolism and drug-drug interactions (DDI); correlations between studies in vitro and in vivo; timing of metabolism studies; labeling; and related applications and considerations. This subject is discussed in detail in Chapter 6 of this book.

"In Vivo Drug Metabolism/Drug Interaction Studies—Study Design, Data Analysis, and Recommendations for Dosing and Labeling" Guidance (1999)

This guidance provides recommendations to sponsors of NDAs and biologies license applications (BLAs) for therapeutic biologies (hereafter drugs) who intend to perform in vivo drug metabolism and metabolic drug-drug interaction studies. The guidance reflects the Agency's current view that the metabolism of an investigational new drug should be defined during drug development and that its interactions with other drugs should be explored as part of an adequate assessment of its safety and effectiveness. For metabolic drug-drug interactions, the approaches considered in the guidance are offered with the understanding that whether a particular study should be performed will vary, depending on the drug in development and its intended clinical use. Furthermore, not every drug-drug interaction is metabolism-based, but may arise from changes in PK caused by absorption, tissue, and/or plasma binding, distribution and excretion interactions. Drug interactions related to transporters or pharmacodynamic-based drug interactions are not covered in this guidance.

After a brief discussion on metabolism and metabolic DDIs, the guidance covers the following topics: general strategies; design of in vivo metabolic drug-drug interaction studies; and labeling.

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"Pharmacokinetics in Patients with Impaired Renal Function—Study Design, Data Analysis, and Impact on Dosing and Labeling" Guidance (1998)

This guidance is intended for sponsors who, during the investigational phase of drug development, plan to conduct studies to assess the influence of renal impairment on the PK of an investigational drug. Topics covered in this guidance are: deciding whether to conduct a study in patients with impaired renal function (when studies may be important, when studies may not be important); study design (basic "full" study design, reduced/staged study design, population PK studies, effect of dialysis on PK, PD assessments); data analysis (parameter estimation, modeling the relationship between renal function and PK, development of dosing recommendations); and labeling (clinical pharmacology, precautions/warnings, dosage and administration, overdosage).

"Population Pharmacokinetics" Guidance (1999)

This guidance makes recommendations on the use of population PK in the drug development process to help identify differences in drug safety and efficacy among population subgroups. It summarizes scientific and regulatory issues that should be addressed using population PK. The guidance discusses when to perform a population PK study and/or analysis; how to design and execute a population PK study; how to handle and analyze population PK data; what model validation methods are available; and how to provide appropriate documentation for population PK reports intended for submission to the FDA.

"Pharmacokinetics in Patients with Impaired Hepatic Function: Study Design, Data Analysis, and Impact on Dosing and Labeling" Guidance (2002)

This guidance provides recommendations to sponsors planning to conduct studies to assess the influence of hepatic impairment on the PK and, where appropriate, PD of drugs or therapeutic biologies. This guidance addresses: when studies are and may not be recommended; the design and conduct of studies to characterize the effects of impaired hepatic function on the PK of a drug; characteristics of patient populations to be studied; and analysis, interpretation, and reporting of the results of the studies and description of the results in labeling.

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Biopharmaceutics

"Bioanalytical Method Validation" Guidance (2001)

This guidance provides assistance to sponsors of INDs, NDAs, AND As, and supplements in developing bioanalytical method validation information used in human clinical pharmacology, BA, and BE studies requiring PK evaluation. This guidance also applies to bioanalytical methods used for nonhuman pharmacology/toxicology studies and preclinical studies. For studies related to the veterinary drug approval process, this guidance applies only to blood and urine BA, BE, and PK studies. The information in this guidance generally applies to bioanalytical procedures such as gas chromatography (GC), high-pressure liquid chromatography (LC), combined GC and LC mass spectrometric (MS) procedures such as LC-MS, LC-MS-MS, GC-MS, and GC-MS-MS performed for the quantitative determination of drugs and/or metabolites in biological matrices such as blood, serum, plasma, or urine. This guidance also applies to other bioanalytical methods, such as immunological and microbiological procedures, and to other biological matrices, such as tissue and skin samples. The guidance touches upon the full, partial, and cross validation and then covers the following topics in detail: reference standard; method development (chemical as well as microbiological and ligand-binding assays); application of validated method to routine drug analysis; and documentation.

"Dissolution Testing of Immediate Release Solid Oral Dosage Forms" Guidance (1997)

This guidance is intended to provide (i) general recommendations for dissolution testing; (ii) approaches for setting dissolution specifications related to the biopharmaceutic characteristics of the drug substance; (iii) statistical methods for comparing dissolution profiles; and (iv) a process to help determine when dissolution testing is sufficient to grant a waiver for an in vivo bioequivalence study. This document also provides recommendations for dissolution tests to help ensure continuous drug product quality and performance after certain postapproval manufacturing changes. Information on dissolution methodology, apparatus, and operating conditions for dissolution testing of IR products is provided in summary form in Appendix A. This guidance is intended to complement the SUPAC—IR guidance for industry (Immediate Release Solid Oral Dosage Forms: Scaleup and Post-Approval Changes: Chemistry, manufacturing and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation) with specific reference to the generation of dissolution profiles for comparative purposes.

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The topics covered in this guidance are: biopharmaceutics classification system; setting dissolution specifications; dissolution profile comparisons; dissolution and SUPAC-IR; and biowaivers.

"Extended Release Oral Dosage Forms: Development, Evaluation, and Application of in vitro/in vivo Correlations" Guidance (1997)

This guidance provides recommendations to pharmaceutical sponsors who intend to develop documentation in support of an in vitro/in vivo correlation (IVIVC) for an oral extended release (ER) drug product for submission in an NDA or ANDA. The guidance presents a comprehensive perspective on (i) methods of developing an IVIVC and evaluating its predictability; (ii) using an IVIVC to set dissolution specifications; and (iii) applying an IVIVC as a surrogate for in vivo bioequivalence when it is necessary to document bioequivalence during the initial approval process or because of certain pre or postapproval changes (e.g., formulation, equipment, process, and manufacturing site changes).

The topics covered in this guidance are: categories of in vitro/in vivo correlations; general considerations; development and evaluation of a level A in vitro/in vivo correlation; development and evaluation of a level C correlation; and applications of an IVIVC.

"Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System" Guidance (2000)

This guidance provides recommendations for sponsors of INDs, NDAs, ANDAs, and supplements to these applications who wish to request a waiver of in vivo BA and/or BE studies for IR solid oral dosage forms. These waivers are intended to apply to (i) subsequent in vivo BA or BE studies of immediate-release (IR) formulations after the initial establishment of in vivo BA during the IND phase and (ii) in vivo BE studies of IR oral dosage forms in ANDAs. In addition to the regulations at 21 CFR 320 that address biowaivers, this guidance explains when biowaivers can be requested for IR solid oral dosage forms based on an approach termed the Biopharmaceutics Classification System (BCS).

The topics covered in this guidance are: the biopharmaceutics classification system; methodology for classifying a drug substance and for determining the dissolution characteristics of a drug product; additional considerations for requesting a biowaiver; regulatory applications of the BCS; and data to support a request for biowaivers.

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"Statistical Approaches to Establishing Bioequivalence" Guidance (2001)

This guidance provides recommendations to sponsors and applicants who intend, either before or after approval, to use equivalence criteria in analyzing in vivo or in vitro BE studies for INDs, NDAs, ANDAs, and supplements to these applications. This guidance discusses three approaches for BE comparisons: average, population, and individual. The guidance focuses on how to use each approach once a specific approach has been chosen. This guidance replaces a prior FDA guidance entitled Statistical Procedures for Bioequivalence Studies Using a Standard Two-Treatment Crossover Design, which was issued in July 1992.

The topics covered in this guidance are: statistical model; statistical approaches for bioequivalence; study design; statistical analysis; and miscellaneous issues.

"Bioavailability and Bioequivalence Studies for Orally Administered Drug Products—General Considerations" Guidance (2000)

This guidance is intended to provide recommendations to sponsors or applicants planning to include BA and BE information for orally administered drug products in the INDs, NDAs, ANDAs, and their supplements. This guidance addresses how to meet the BA and BE requirements set forth in 21 CFR 320 as they apply to dosage forms intended for oral administration. These include tablets, capsules, solutions, suspensions, conventional/immediate release, and modified (extended/delayed) release drug products. The guidance is also generally applicable to nonorally administered drug products where reliance on systemic exposure measures is suitable to document BA and BE (e.g., transdermal delivery systems and certain rectal and nasal drug products).

This guidance starts with the definitions and a detailed discussion of the terms BA and BE which is then followed by a discussion on the following topics: methods to document BA and BE; comparison of BA measures in BE studies; documentation of BA and BE; and special topics namely food-effect studies, moieties to be measured, long half-life drugs, first point Cmax, orally administered drugs intended for local action and narrow therapeutic range drugs.

This guidance is designed to reduce the need for FDA drug-specific BA/BE guidances. As a result, this guidance replaces a number of previously issued FDA drug-specific guidances which are listed in the Appendix 1 of this guidance.

A concluding remark on the U.S. regulations and guidances is that there are a few pertinent guidances which are at the draft stage that are not

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covered in this chapter and the reader is strongly encouraged to get familiar with them and follow their progress till issuance of the final version. Probably the most critical ones are the "Exposure-Response" and the "Food-Effect" guidances.

ICH GUIDELINES

With the globalization of the pharmaceutical industry, efforts have been underway since 1990 to standardize drug applications in terms of content and format such that an application can be registered in different countries without being subjected to different registration requirements among countries. Via efforts that include the participation of the European Union, Japan, and the United States, ICH guidelines have been prepared or are in the process of being finalized on the topics of Quality (the Q series of guidelines), Safety (the S series of guidelines), Efficacy (the E series of guidelines), and Multidisciplinary (the M series of guidelines). Care has been taken while reaching consensus with the other world bodies that the information that is needed is based on U.S. laws and CFR regulations plus similar considerations for the other world regulatory agencies. Relevant ICH guidelines [http://www.ifpma.org/ichl] as related to this chapter which are either completed or at advanced stages of completion (step 4) are covered.[6]

The order of presentation of these guidelines is based on their completion dates (earliest to latest) and not the sequence number given by the ICH (e.g., E3 followed by E4, etc.). The reason is that it appears that clinical pharmacology and biopharmaceutic concepts, and related recommendations, got introduced in the earliest guidelines in a broad and diffused sense and they subsequently got elaborated upon and covered in more detail in later guidelines.

E7: "Studies in Support of Special Populations: Geriatrics" Guideline (1993)

As stated earlier, it appears that this guideline is modeled after an updated version of, the U.S. "elderly" guidance of 1989. It covers PK studies (formal or a PK screen) in the elderly as well as renally or hepatically impaired patients, PD/Dose-response studies and drug-drug interaction studies as follows.

Pharmacokinetic Studies

The guideline states that most of the recognized important differences between younger and older patients have been pharmacokinetic differences,

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often related to impairment of excretory (renal or hepatic) function or to drug-drug interactions. It is important to determine whether or not the pharmacokinetic behavior of the drug in elderly subjects or patients is different from that in younger adults and to characterize the effects of influences, such as abnormal renal or hepatic function, that are more common in the elderly even though they can occur in any age group. Information regarding age-related differences in the pharmacokinetics of the drug can come, at the sponsor's option, either from a Pharmacokinetic Screen or from formal pharmacokinetic studies, in the elderly and in patients with excretory functional impairment.

The guideline recognizes that for certain drugs and applications (e.g., some topically applied agents, some proteins) technical limitations such as low systemic drug levels may preclude or limit exploration of age-related pharmacokinetic differences.

Pharmacokinetics in Renally or Hepatically Impaired Patients

As stated in the guideline, renal impairment is an aging-associated finding that can also occur in younger patients. Therefore, it is a general principle that drugs excreted (parent drug or active metabolites) significantly through renal mechanisms should be studied to define the effects of altered renal function on their pharmacokinetics. Such information is needed for drugs that are the subject of this guideline but it can be obtained in younger subjects with renal impairment.

Similarly, drugs subject to significant hepatic metabolism and/or excretion, or that have active metabolites, may pose special problems in the elderly. Pharmacokinetic studies should be carried out in hepatically impaired young or elderly patient volunteers.

If a Pharmacokinetic Screen approach is chosen by the sponsor, and if patients with documented renal impairment or hepatic impairment (depending on the drug's elimination pattern) are included and the results indicate no medically important pharmacokinetic difference, that information may be sufficient to meet this geriatric guideline's purpose.

Pharmacodynamic/Dose Response Studies

The guideline states that the number of age-related pharmacodynamic differences (i.e., increased or decreased therapeutic response, or side effects, at a given plasma concentration of drug) discovered to date is too small to necessitate dose response or other pharmacodynamic studies in geriatric patients as a routine requirement. Separate studies are, however, recommended in the following situations:

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- Sedative/hypnotic agents and other psychoactive drugs or drugs with important CNS effects, such as sedating antihistamines.
- Where subgroup comparisons (geriatric versus younger) in the Phase 2/3 clinical trials database indicate potentially medically significant age-associated differences in the drug's effectiveness or adverse reaction profile, not explainable by PK differences.

Drug-Drug Interaction Studies

As per the guideline, such interactions are of particular importance to geriatric patients, who are more likely to be using concomitant medications than younger patients, but of course are not limited to this age group. Therefore it is a general principle, not specific to these guidelines, that in cases where the therapeutic range (i.e., a range of toxic to therapeutic doses) of the drug or likely concomitant drugs is narrow, and the likelihood of the concomitant therapy is great, that specific drug-drug interaction studies be considered. The studies needed must be determined case-by-case, but the following are ordinarily recommended:

- Digoxin and oral anticoagulant interaction studies, because so many drugs alter serum concentrations of these drugs, they are widely prescribed in the elderly, and they have narrow therapeutic ranges.
- For drugs that undergo extensive hepatic metabolism, determination of the effects of hepatic-enzyme inducers (e.g., phenobarbital) and inhibitors (e.g., cimetidine).
- For drugs metabolized by cytochrome P-450 enzymes, it is critical to examine the effects of known inhibitors, such as quinidine (for cytochrome P-450 2D6) or ketoconazole and macrolide antibiotics (for drugs metabolized by cytochrome P-450 3A4). There is a rapidly growing list of drugs that can interfere with other drugs via metabolism, and sponsors should remain aware of it.
- Interaction studies with other drugs that are likely to be used with the test drug (unless important interactions have been ruled out by a Pharmacokinetic Screen).

E4: "Dose-Response Information to Support Drug Registration" Guideline (Step 4; 1994)

This guideline covers the following topics: (i) introduction (purpose of doseresponse information, use of dose-response information in choosing doses, use of concentration-response data, problems with titration designs, interaction between dose-response and time), (ii) obtaining dose-response

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information (dose-response assessment should be an integral part of drug development, studies in life-threatening diseases, regulatory considerations when dose-response data are imperfect, examining the entire database for dose-response information), (iii) study designs for assessing dose-response (general, specific trial designs), and (iv) guidance and advice.

The reader is strongly encouraged to read this guideline since it lays out the fundamental value and benefit of the exposure (i.e., dose and/or concentration)—response information in drug development and evaluation, and recognizes past inadequacies as well as practical limitations in generation of this information base. As per the guideline, where a drug can be safely and effectively given only with blood concentration monitoring, the value of concentration-response information is obvious. In other cases, an established concentration-response relationship is often not needed, but may be useful for ascertaining the magnitude of the clinical consequences of (i) pharmacokinetic differences, such as those due to drug-disease (e.g., renal failure) or drug-drug interactions, or (ii) for assessing the effects of the altered pharmacokinetics of new dosage forms (e.g., controlled release formulation) or new dosage regimens without need for additional clinical data, where such assessment is permitted by regional regulations. Prospective randomized concentration-response studies are critical to defining concentration monitoring therapeutic "windows" but are also useful when pharmacokinetic variability among patients is great; in this case, a concentration-response relationship may in principle be discerned in a prospective study with a smaller number of subjects than could be the dose response relationship in a standard dose-response study. Note that collection of concentration-response information does not imply that therapeutic blood level monitoring will be needed to administer the drug properly. Concentration-response relationships can be translated into doseresponse information. Alternatively, if the relationships between concentration and observed effects (e.g., an undesirable or desirable pharmacologic effect) are defined, patient response can be titrated without the need for further blood level monitoring. Concentration-response information can also allow selection of doses (based on the range of concentrations they will achieve) most likely to lead to a satisfactory response.

E3: "Structure and Content of Clinical Study Reports" Guideline (Step 4; 1995)

The relevant portions of this guideline from a clinical pharmacology perspective are the sections which cover the "drug concentration measurements," "drug dose, drug concentration, and relationships to response," and "drug-drug and drug-disease interactions" topics.

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Further discussion of this guideline is not undertaken in this chapter since these topics are also covered in other guidelines, particularly the M4 guideline discussed later in this chapter.

E8: "General Considerations for Clinical Trials" Guideline (1997)

This guideline goes over general principles of clinical trials in terms of protection of subjects and scientific approach in design and analysis, as well as development methodology in terms of considerations for the development plan and considerations for individual clinical trials.

A very informative section in this guideline is Table 1 that provides an approach to classifying clinical studies according to objectives. The table breaks down the types of studies into four categories, namely Human Pharmacology, Therapeutic Exploratory, Therapeutic Confirmatory, and Therapeutic Use and lists the objectives of such studies along with examples. The first two categories of studies identify clinical pharmacology studies. The Human Pharmacology category comprises studies that assess tolerance, define/describe PK and PD, explore drug metabolism and drug interactions, and enzyme activity. Examples of such studies are dose-tolerance studies, single and multiple dose PK and/or PD studies, and drug interaction studies. Similarly, the Therapeutic Exploratory category consists of studies that explore use for the targeted indication, estimate dosage for subsequent studies, provide basis for confirmatory study design, endpoints, and methodologies. Examples of such studies are the earliest trials of relatively short duration in well-defined narrow patient populations, using surrogate or pharmacological endpoints of clinical measures, and dose-response exploration studies.

Additional sections outlining clinical pharmacology and biopharmaceutic considerations are:

- Quality of investigational medicinal products
- Phase I (Most typical kind of study: human pharmacology)
  - Estimation of initial safety and tolerability
  - Pharmacokinetics
  - Assessment of pharmacodynamics
  - Early measurement of drug activity
- Special considerations
  - Studies of drug metabolites
  - Drug-drug interactions
  - Special populations
  - Investigations in nursing women

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E5: "Ethnic Factors in the Acceptability of Foreign Clinical Data" Guideline (Step 4; 1998)

This guideline is based on the premise that it is not necessary to repeat an entire clinical drug development program in a new region, and it is intended to recommend strategies for accepting foreign clinical data as full or partial support for approval of an application in a new region. It is a strong endorsement of the utility of clinical pharmacology information. A couple of key concepts—bridging study and compounds sensitive to ethnic factors—in this guideline are based on, or utilize, clinical pharmacology information. Additionally, it also provides a definition of a PK study, a PD study, and Population PK Methods as well as providing a good discussion of PK, PD, and dose-response considerations.

Bridging Study

A bridging study is defined as a supplemental study performed in the new region to provide pharmacodynamic or clinical data on efficacy, safety, dosage, and dose regimen in the new region that will allow extrapolation of the foreign clinical data to the new region. Such studies could include additional pharmacokinetic information.

Compounds Sensitive to Ethnic Factors

A compound who's pharmacokinetic, pharmacodynamic, or other characteristics suggest the potential for clinically significant impact by intrinsic and/or extrinsic ethnic factors [covered further in the M4 guideline] on safety, efficacy, or dose response.

Pharmacokinetic Study

A study of how a medicine is handled by the body, usually involving measurement of blood concentrations of drug and its metabolite(s) (sometimes concentrations in urine or tissues) as a function of time. Pharmacokinetic studies are used to characterize absorption, distribution, metabolism, and excretion of a drug, either in blood or in other pertinent locations. When combined with pharmacodynamic measures (a PK/PD study) it can characterize the relation of blood concentrations to the extent and timing of pharmacodynamic effects.

Pharmacodynamic Study

A study of a pharmacological or clinical effect of the medicine in individuals to describe the relation of the effect to dose or drug concentration. A pharmacodynamic effect can be a potentially adverse effect (anticholinergic effect with a tricyclic), a measure of activity thought related to clinical

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benefit (various measures of beta-blockade, effect on ECG intervals, inhibition of ACE or angiotensin I or II response), a short-term desired effect, often a surrogate endpoint (blood pressure, cholesterol), or the ultimate intended clinical benefit (effects on pain, depression, sudden death).

Population Pharmacokinetic Methods

Population pharmacokinetic methods are a population-based evaluation of measurements of systemic drug concentrations, usually two or more per patient under steady state conditions, from all, or a defined subset of, patients who participate in clinical trials.

Pharmacokinetic, Pharmacodynamic, and Dose Response Considerations

Evaluation of the pharmacokinetics and pharmacodynamics, and their comparability, in the three major racial groups most relevant to the ICH regions (Asian, Black, and Caucasian) is critical to the registration of medicines in the ICH regions. Basic pharmacokinetic evaluation should characterize absorption, distribution, metabolism, excretion (ADME), and where appropriate, food-drug and drug-drug interactions. Adequate pharmacokinetic comparison between populations of different regions allows rational consideration of what kinds of further pharmacodynamic and clinical studies (bridging studies) are needed for the new region. In contrast to the pharmacokinetics of a medication, where differences between populations may be attributed primarily to intrinsic ethnic factors and are readily identified, the pharmacodynamic response (clinical effectiveness, safety, and dose-response) may be influenced by both intrinsic and extrinsic ethnic factors and this may be difficult to identify except by conducting clinical studies in the new region.

In general, dose-response (or concentration-response) should be evaluated for both pharmacologic effect (where one is considered pertinent) and clinical endpoints in a new foreign region. The pharmacologic effect, including dose-response, may also be evaluated in the foreign region in a population representative of the new region.

Depending on the situation, data on clinical efficacy and doseresponse in the new region may or may not be needed, e.g., if the drug class is familiar and the pharmacologic effect is closely linked to clinical effectiveness and dose-response, the foreign pharmacodynamic data may be a sufficient basis for approval and clinical endpoint and dose-response data may not be needed in the new region. The pharmacodynamic evaluation, and possible clinical evaluation (including dose-response), is important because of the possibility that the response curve may be shifted in a new population.

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Examples of this are well documented, e.g., the decreased response in blood pressure of blacks to angiotensin-converting enzyme inhibitors.

E11: "Clinical Investigations of Medicinal Products in the Pediatric Population" Guideline (2000)

The sections of this guideline that outline the clinical pharmacology information are:

Types of Studies

When a medicinal product is to be used in the pediatric population for the same indication(s) as those studied and approved in adults, the disease process is similar in adults and pediatric patients, and the outcome of therapy is likely to be comparable, therefore extrapolation from adult efficacy data may be appropriate. In such cases, pharmacokinetic studies in all the age ranges of pediatric patients likely to receive the medicinal product, together with safety studies, may provide adequate information for use by allowing selection of pediatric doses that will produce blood levels similar to those observed in adults. If this approach is taken, adult pharmacokinetic data should be available to plan the pediatric studies.

When a medicinal product is to be used in younger pediatric patients for the same indication(s) as those studied in older pediatric patients, the disease process is similar, and the outcome of therapy is likely to be comparable, therefore extrapolation of efficacy from older to younger pediatric patients may be possible. In such cases, pharmacokinetic studies in the relevant age groups of pediatric patients likely to receive the medicinal product, together with safety studies, may be sufficient to provide adequate information for pediatric use.

An approach based on pharmacokinetics is likely to be insufficient for medicinal products where blood levels are known or expected not to correspond with efficacy, or where there is concern that the concentrationresponse relationship may differ between the adult and pediatric populations. In such cases, studies of the clinical or the pharmacological effect of the medicinal product would usually be expected.

Where the comparability of the disease course or outcome of therapy in pediatric patients is expected to be similar to adults, but the appropriate blood levels are not clear, it may be possible to use measurements of a pharmacodynamic effect related to clinical effectiveness to confirm the expectations of effectiveness and to define the dose and concentration needed to attain that pharmacodynamic effect. Such studies could provide increased confidence that achieving a given exposure to the medicinal product in pediatric patients would result in the desired therapeutic

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outcomes. Thus, a PK/PD approach combined with safety and other relevant studies could avoid the need for clinical efficacy studies.

In other situations where a pharmacokinetic approach is not applicable, such as for topically active products, extrapolation of efficacy from one patient population to another may be based on studies that include pharmacodynamic endpoints and/or appropriate alternative assessments. Local tolerability studies may be needed. It may be important to determine blood levels and systemic effects to assess safety.

Pharmacokinetics

Pharmacokinetic studies generally should be performed to support formulation development and determine pharmacokinetic parameters in different age groups to support dosing recommendations. Relative bioavailability comparisons of pediatric formulations with the adult oral formulation typically should be done in adults. Definitive pharmacokinetic studies for dose selection across the age ranges of pediatric patients in whom the medicinal product is likely to be used should be conducted in the pediatric population.

For medicinal products that exhibit linear pharmacokinetics in adults, single-dose pharmacokinetic studies in the pediatric population may provide sufficient information for dosage selection. This can be corroborated, if indicated, by sparse sampling in multidose clinical studies. Any nonlinearity in absorption, distribution, and elimination in adults and any difference in duration of effect between single and repeated dosing in adults would suggest the need for steady state studies in the pediatric population. All these approaches are facilitated by knowledge of adult pharmacokinetic parameters. Knowing the pathways of clearance (renal and metabolic) of the medicinal product and understanding the age-related changes of those processes will often be helpful in planning pediatric studies.

M4: "The Common Technical Document for the Registration of Pharmaceuticals for Human Use. EFFICACY. Module 2: Clinical Overview and Clinical Summary. Module 5: Clinical Study Reports" (Step 4; 2000)

This is a very comprehensive guideline that identifies all important aspects of clinical pharmacology and biopharmaceutic considerations and provides details on format and content of related requirements. In view of the authors, this is a comprehensive update of the United States guideline issued in 1987 and is a must-read.

As stated in the title, module 2 in this guideline goes over the organization and content of the clinical overview and the clinical summary sections.

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Following this, module 5 provides organization of clinical study reports and related information. These reports are broken down into seven different categories: Biopharmaceutics Studies; Studies Pertinent to PK Using Human Biomaterials; Human PK Studies; Human PD Studies; Efficacy and Safety Studies; Postmarketing Experience; Case Report Forms; and Individual Patient Listings. The first four of these report types form the basis for clinical pharmacology and biopharmaceutics information required in an application and are covered in detail below:

Biopharmaceutic Studies

This guideline states that bioavailability studies evaluate the rate and extent of release of the active substance from the medicinal product. Comparative BA or BE studies may use PK, PD, clinical, or in vitro dissolution endpoints, and may be either single dose or multiple dose. Types of BA studies identified are (i) studies comparing the release and systemic availability of a drug substance from a solid oral dosage form to the systemic availability of the drug substance given intravenously or as an oral liquid dosage form, (ii) dosage form proportionality studies, and (iii) food-effect studies. Next set of studies identified are comparative BA and BE studies, and these are studies that compare the rate and extent of release of the drug substance from similar drug products (e.g., tablet to tablet, tablet to capsule). Comparative BA or BE studies may include comparisons between (i) the drug product used in clinical studies supporting effectiveness and the to-be-marketed drug product, (ii) the drug product used in clinical studies supporting effectiveness and the drug product used in stability batches, and (iii) similar drug products from different manufacturers. The final type of studies identified are In Vitro—In Vivo Correlation studies, i.e., in vitro dissolution studies that provide BA information, including studies used in seeking to correlate in vitro data with in vivo performance.

Studies Pertinent to Pharmacokinetics Using Human Biomaterials

The guideline defines human biomaterials as proteins, cells, tissues, and related materials derived from human sources, which are used in vitro or ex vivo to assess PK properties of drug substances. The types of studies identified are plasma protein binding studies, and hepatic metabolism and drug interaction studies. Examples include cultured human colonic cells that are used to assess permeability through biological membranes and transport processes, and human albumin that is used to assess plasma protein binding. Of particular importance is the use of human biomaterials such as hepatocytes and/or hepatic microsomes to study metabolic pathways and to assess drug-drug interactions with these pathways.

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Human Pharmacokinetic Studies

According to the guideline, assessment of the PK of a drug in healthy subjects and/or patients is considered critical to designing dosing strategies and titration steps, to anticipating the effects of concomitant drug use, and to interpreting observed pharmacodynamic differences. These assessments should provide a description of the body's handling of a drug over time, focusing on maximum plasma concentrations (peak exposure), area-undercurve (total exposure), clearance, and accumulation of the parent drug and its metabolite(s), in particular those that have pharmacological activity. The PK studies are generally designed to (i) measure plasma drug and metabolite concentrations over time, (ii) measure drug and metabolite concentrations in urine or feces when useful or necessary, and/or (iii) measure drug and metabolite binding to protein or red blood cells.

On occasion, PK studies may include measurement of drug distribution into other body tissues, body organs, or fluids (e.g., synovial fluid or cerebrospinal fluid). These studies should characterize the drug's PK and provide information about the absorption, distribution, metabolism, and excretion of a drug and any active metabolites in healthy subjects and/or patients. Studies of mass balance and changes in PK related to dose (e.g., determination of dose proportionality) or time (e.g., due to enzyme induction or formation of antibodies) are of particular interest. Additional studies can also assess differences in systemic exposure as a result of changes in PK due to intrinsic (e.g., age, gender, racial, weight, height, disease, genetic polymorphism, and organ dysfunction) and extrinsic (e.g., drug-drug interactions, diet, smoking, and alcohol use) factors. In addition to standard multiple-sample PK studies, population PK analyses based on sparse sampling during clinical studies can also address questions about the contributions of intrinsic and extrinsic factors to the variability in the dosePK-response relationship. Thus, the guideline identifies the following types of studies as Human PK studies: Healthy subject PK and initial tolerability; Patient PK and initial tolerability; Intrinsic factor PK; Extrinsic factor PK; and Population PK.

Human Pharmacodynamic Studies

The guideline identifies these as (i) studies of pharmacologic properties known or thought to be related to the desired clinical effects (biomarkers), (ii) short-term studies of the main clinical effect, and (iii) PD studies of other properties not related to the desired clinical effect. Because a quantitative relationship of these pharmacological effects to dose and/or plasma drug and metabolite concentrations is usually of interest, PD information is frequently collected in dose response studies or together with drug

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concentration information in PK studies (concentration-response or PK/PD studies). The guideline states that dose-finding, PD and/or PK-PD studies can be conducted in healthy subjects and/or patients, and can also be incorporated into the studies that evaluate safety and efficacy in a clinical indication. In some cases, the short-term PD, dose-finding, and/or PK-PD information found in pharmacodynamic studies conducted in patients will provide data that contribute to assessment of efficacy, either because they show an effect on an acceptable surrogate marker (e.g., blood pressure) or on a clinical benefit endpoint (e.g., pain relief). Thus the studies identified here are healthy subject PD and PK/PD studies plus patient PD and PK/PD studies.

The reader must note that the guideline clearly states that when these PD studies are part of the efficacy or safety demonstration, they are considered clinical efficacy and safety studies that should be included in Section 5. Similarly, studies whose primary objective is to establish efficacy or to accumulate safety should be included in Section 5.

Section 5 is beyond the scope of this chapter.

GLOSSARY

Bioavailability. The rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available to the site of action.

Bioeqivalence. The absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study. Where there is an intentional difference in rate (e.g., in certain controlled release dosage forms), certain pharmaceutical equivalents or alternatives may be considered bioequivalent if there is no significant difference in the extent to which the active ingredient or moiety from each product becomes available at the site of drug action. This applies only if the difference in the rate at which the active ingredient or moiety becomes available at the site of drug action is intentional, is reflected in the proposed labeling, is not essential to the attainment of effective body drug concentrations on chronic use, and is considered medically insignificant for the drug.

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Drug. Means (i) articles recognized in the official *United States Pharmacopoeia,* official *Homoeopathic Pharmacopoeia of the United States,* or official *National Formulary,* or any supplement to any of them; and (ii) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and (iii) articles (other than food) intended to affect the structure or any function of the body of man or other animals; and (iv) articles intended for use as a component of any article specified in clause (i), (ii), or (iii); but does not include devices or their components, parts, or accessories.

Drug Product. A finished dosage form, e.g., tablet, capsule, or solution, that contains the active drug ingredient, generally, but not necessarily, in association with the inactive ingredients.

Extended Release. Extended release products are formulated to make the drug available over an extended period after ingestion. This allows a reduction in dosing frequency compared to a drug presented as a conventional dosage form (e.g., as a solution or an immediate release dosage form).

Immediate Release. Allows the drug to dissolve in the gastrointestinal contents, with no intention of delaying or prolonging the dissolution or absorption of the drug.

Interstate Commerce. Means (i) commerce between any State or Territory and any place outside thereof, and (ii) commerce within the District of Columbia or within any other Territory not organized with a legislative body.

Labeling. All labels and other written, printed, or graphic matter (i) upon any article or any of its containers or wrappers, or (ii) accompanying such article.

Modified Release Dosage Forms. Dosage forms whose drug-release characteristics of time course and/or location are chosen to accomplish therapeutic or convenience objectives not offered by conventional dosage forms such as a solution or an immediate release dosage form. Modified release solid oral dosage forms include both delayed and extended release drug products.

Pharmaceutical Alternatives. Drug products that contain the identical therapeutic moiety, or its precursor, but not necessarily in the same amount or dosage form or as the same salt or ester. Each such drug product individually meets either the identical or its own respective compendial or other applicable standard of identity, strength, quality, and purity, including

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potency and, where applicable, content uniformity, disintegration times, and/or dissolution rates.

Pharmaceutical Equivalents. Drug products that contain identical amounts of the identical active drug ingredient, i.e., the same salt or ester of the same therapeutic moiety, in identical dosage forms, but not necessarily containing the same inactive ingredients and that meet the identical compendial or other applicable standards of identity, strength, quality, and purity, including potency and, where applicable, content uniformity, disintegration times and/or dissolution rates.

ACKNOWLEDGMENT

The authors thank Mr. Donald Hare for his useful suggestions and input.

NOTES

1. The text of the Federal Food, Drug, and Cosmetic Act, as amended, can be found codified in the United States Code (USC) under Title 21 (Food and Drugs). Example, FDCA Section 505 for New Drugs can also be found in Section 355 of Title 21 of USC (21 USC 355).
2. As a result of the disaster where it was discovered that the drug thalidomide caused deformities in newborn children, the Kefauver-Harris Amendments were added to the FDCA in 1962. These amendments covered or required that (i) efficacy in addition to safety be demonstrated for a product, (ii) there be good manufacturing practices (GMPs) for which products could be removed from the market if not manufactured in conformity with current good manufacturing practices (CGMPs) to ensure product quality, (iii) there be implementation of investigational new drug applications (INDs), and (iv) prescription drug advertising be put under FDA supervision while advertising for over-the-counter (OTC) products would remain with the Federal Trade Commission (FTC).
3. It is noted that all products that are approved via 505(b)(1) or 505(b)(2) applications or as supplements to NDAs, if appropriate, are also included in the Orange Book and are coded as appropriate among the different codes that are allowed.
4. Before a rule or regulation is codified in the CFR, it is published as a proposed rule or regulation in the FR for which public comment is requested and after which it is finalized in a subsequent FR publication with modifications if needed. In the CFR, relevant FR publications are usually referenced. The FR and CFR can be accessed via the internet at http://www.access.gpo.gov/su_docs/index.html.
5. Before a guidance is finalized, it is published as a draft in the FR in order to

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obtain public comment. The finalized guidance is published in a subsequent FR notice.

6. There are five steps in the ICH process of guideline development and issuance which are Consensus Building (Step 1), Start of Regulatory Action (Step 2), Regulatory Consultation (Step 3), Adoption of a Tripartite Harmonized Text (step 4), and Implementation (Step 5).

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4

New Drug Application Content and Review Process for Clinical Pharmacology and Biopharmaceutics

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INTRODUCTION

The regulation and control of new drugs in the United States has been based on the new drug application (NDA) that is evaluated by the U.S. Food and Drug Administration (FDA). The data gathered in preclinical studies and human clinical trials as an investigational new drug (IND) during the drug development process become part of the NDA. The goal of the drug development process is to provide sufficient information to the FDA in the NDA to evaluate the efficacy and safety of the new drug as well as recommendations to adjust the dose in special circumstances. The drug development process for new drugs has evolved over the years especially in the field of Clinical Pharmacology and Biopharmaceutics. In response to the

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evolving technology, advancement of knowledge in the field, and to ascertain consistency and quality of the data available during the development process, the US FDA, including, office of clinical pharmacology and biopharmaceutics (OCPB) has issued several regulatory guidance documents. Office of clinical pharmacology and biopharmaceutics has several guidances in the public domain that are available to drug companies (often referred to as sponsors) which provide recommendations in the areas of clinical pharmacology/ biopharmaceutics such as exposure-response assessments, design and conduct of population pharmacokinetic studies, in vitro and in vivo drug metabolism and drug interactions, dissolution testing requirements for immediate and extended release dosage forms, design and conduct of bioavailability, bioequivalence and food-effect studies, and studies in patients with renal and hepatic impairment. This chapter integrates the information from available OCPB and other FDA-issued guidances that aid in the drug development process, and also provides insight into some of the issues that should be considered from a regulatory perspective regarding the Clinical Pharmacology and Biopharmaceutics aspects of drug development. It should be noted that some of the guidances are published as a draft and reflect current scientific understanding and thinking of the FDA scientist.

The sponsors now have option submitting new drug application in NDA format or Common Technical Document (CTD) format. Common technical document format is a format in which clinical, pharmacology/ toxicology and manufacturing data can be submitted to obtain marketing authorization for new drugs in the United States, European Union, and Japan. It should however be noted that CTD and NDA do not differ in the content of the information but mainly the format in which data should be provided.

This chapter provides an insight into the review process by the Clinical Pharmacology and Biopharmaceutics staff.

STAGES IN DRUG DEVELOPMENT AND REGULATORY PROCESS

Once the sponsor has identified a lead compound, traditionally, the drug development process follows a plan. Most pharmaceutical companies have a drug development plan that is unique to their company based on their own experiences. In general all pharmaceutical companies proceed with development to answer several questions about the drug, i.e., is the drug safe, up to what dose or exposure it is safe, how should the dose be adjusted in certain specific populations or when co-administered with other drugs to have optimized formulation for delivery of the drug.

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When a compound has been identified, a Pre-IND (IND-investigational new drug) meeting is occasionally requested with the FDA by sponsors. Sponsor may be a pharmaceutical company or individual investigators. Prior to the meeting, the sponsor usually submits a Pre-IND package. The Pre-IND package may include summary of preclinical data and a concept sheet of a study protocol in order to obtain scientific input from the FDA reviewers regarding the initial IND. The FDA review team consists of a Medical Officer, Clinical Pharmacologist and Pharmacokineticist, Chemist, Pharmacologist/Toxicologist Statistician, and a Microbiologist (depending on the proposed indication). Input requested by the sponsor before the filing of the initial IND usually involves questions regarding appropriate dose and/or dosing regimen selection, safety parameters to be assessed, sampling times (pharmacokinetics and safety), etc., for the "first time in humans" study. Generally, the first study conducted in human volunteers is a clinical pharmacology study to evaluate the safety and pharmacokinetics/pharmacodynamics of the drug in healthy volunteers or, in some cases, patients. Prior to conducting this first-time-in-humans study, the FDA requires the sponsor to have conducted adequate preclinical studies to support such a study. The sponsor may also request FDA input regarding the development plan for their compound, generally if human data on the drug is available from studies conducted outside the USA. In this case, the OCPB reviewer would review the sponsor's plan and provide additional suggestions, whenever necessary. Examples of OCPB input at the Pre-IND stage regarding overall drug development include formulation development plans, dissolution method development, exploring mechanisms of action, design and conduct of in vitro metabolism studies, clinical pharmacology study designs, identifying potentially useful biomarkers, proof of concept and doseranging studies, exposure-response and/or population pharmacokineticpharmacodynamic assessments, as well as design and dose selection plans for Phase 3 studies. Depending on the complexity of the Pre-IND, the Agency would respond either via a letter or a meeting may be set up with the sponsor.

Protocols for all studies conducted in human volunteers in the United States or that would become part of the NDA have to be submitted to the FDA. Once an IND has been filed FDA assigns a number to the IND. Subsequent study protocols, study reports or sponsor's correspondences have to refer to the IND number.

Once the sponsor has submitted an IND to the FDA, FDA has 30 days to review the submitted protocol for human study. During this review, if there are any concerns about the safety of the subjects to be enrolled in the study, FDA would call the sponsor and place the protocol on clinical hold until the concerns identified by the FDA reviewers are satisfactorily addressed. The IND review process is shown schematically in Fig. 1.

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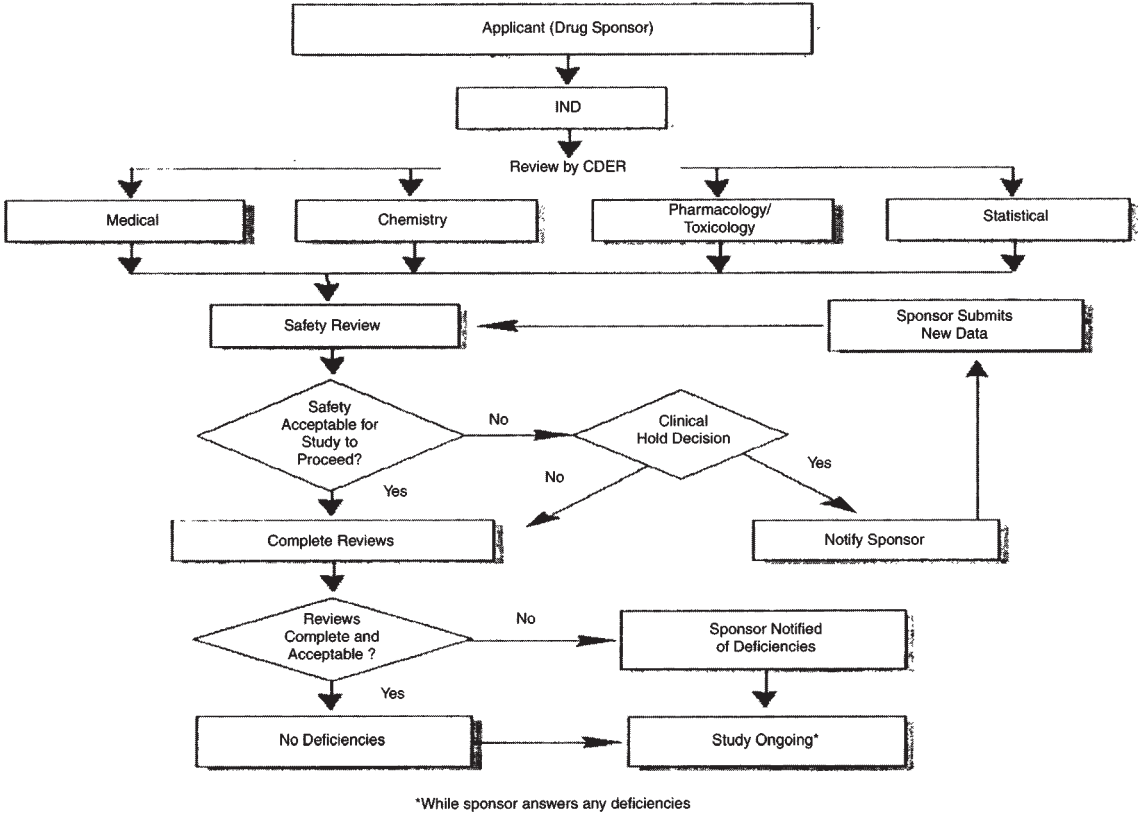

FIGURE 1 The IND review process, http://www.fda.gov/cder.

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There is keen interest on the part of the pharmaceutical companies to be involved in screening INDs (at the time of the initial IND submission) in which several drugs are screened at the same time and one of the compounds is identified for further development. Further details of this approach can be found in manual of policy and procedures (MAPP) on the FDA website [1].

The drug development stages are not rigid, that is, several phases of early drug development (traditionally called Phase 1 and 2 studies) are generally on going simultaneously. Typically, Phase 1 studies are in healthy volunteers, Phase 2 are studies in small numbers of patients, and Phase 3 are larger clinical trials with adequate number of subjects to determine safety and efficacy of the drug. Phase 1 studies typically include studies related to formulation development, assessment of metabolic pathways, assessment of effects of extrinsic and intrinsic factors such as age, gender, disease, other drugs and food, and assessment of PK—PD. Phase 2 studies are typically dose-ranging and proof of concept studies in a small number of patients who comprise the target population (traditionally called Phase 2A). Assessment of PK-PD is also performed in these studies to help provide an understanding of the doses and dose regimens to be further studied. These studies provide the sponsor as well as the regulatory agencies with the type of knowledge about the drug that is needed to design appropriate confirmatory or definitive large clinical trials in the target patient population (traditionally known as Phase 3 trials). Generally, the FDA needs two positive adequately well controlled Phase 3 trials that support the safety and efficacy of the drug in the target population prior to approval for marketing in the U.S. The overall drug development stages are shown schematically in Fig. 2.

Prior to the start of definitive efficacy or Phase 3 trials, the sponsor usually requests to meet with the FDA at an End-of-Phase 2 meeting. At this meeting, the sponsor discusses with the Agency the information that has been learned about the clinical pharmacology and the limited information obtained in patients about the safety and efficacy of the drug. End-of-Phase 2 meeting discussions with the FDA usually revolve around the decision as to whether the sponsor should proceed to conduct the larger Phase 3 trials and, if so, the appropriate study design for these larger Phase 3 studies. Clinical trial simulations using the in vitro and in vivo data collected from the early phases of development may also aid in optimal design of the Phase 3 trials. The sponsor can request a special protocol assessment [1] for evaluating issues related to the adequacy (e.g., design, conduct, analysis) of certain proposed studies associated with the development of their drug products. Three types of protocols are eligible for this special protocol assessment: (1) animal carcinogenicity protocols, (2) final product stability protocols, and (3) clinical protocols of Phase 3 trails whose data will form

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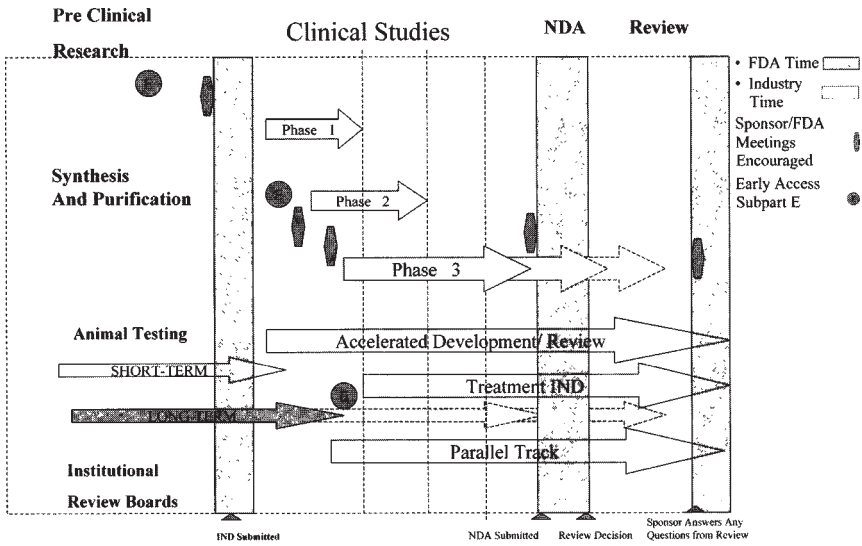

FIGURE 2 Stages in drug development and regulatory process. http://www.fda.gov/cder.

the primary basis for an efficacy claim (if the trials had been discussed at an End-of-Phase 2/pre-Phase 3 meeting or if the review division is aware of the developmental context in which the protocol is being reviewed). The FDA has 45 days to review the protocol and provide scientific/regulatory comments to the sponsor as needed [2]. The guidance recommends that a sponsor submit a protocol intended for special protocol assessment to the Agency at least 90 days prior to anticipated commencement of the study. The protocol should be complete and sufficient time should be allowed to discuss and resolve any issues before the study begins. Special protocol assessments are not to be provided after a study has begun.

There is also a keen interest on the part of the sponsors and the FDA to have a pre-Phase 2 meeting (Phase 2A meeting; i.e., prior to starting the pivotal Phase 2 study in a small set of patients). During this meeting, information available on preclinical studies and Phase 1 studies conducted up to that time can be integrated to assess and discuss Phase 2 protocols. These meetings could provide great opportunity to discuss dosing rationale for the Phase 2 trials, evaluation of appropriate biomarkers, and assessment of exposure-response relationships. There is great interest in these early interactions between the sponsor and the FDA because resources can be used more efficiently and effectively by early communications. There is great

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opportunity for the sponsor and FDA to identify any limitations in the drug development plan early on, so that all relevant information is available at the time NDA/CTD is submitted to the FDA. These meetings have potential to reduce number of review cycles that some times result, and to produce a better drug product label.

Data and information from all studies conducted during the IND phase are summarized and submitted in one package, i.e., NDA. Prior to submission of the NDA, generally the sponsor requests the FDA for a face-to-face Pre-NDA meeting (usually a few months prior to the submission of the NDA). Issues discussed during this meeting include the content and format of the different sections of the NDA that would be considered "fileable," including issues related to electronic submission of the NDA. At this meeting, assessment is also made if any critical piece essential for regulatory decision-making is missing. The FDA has issued a guidance to the industry on the format and content of electronic submissions that are made to the Agency and are available on the FDA Website.

Once an NDA is submitted to the FDA, the agency assigns an NDA number to the drug. Since not all drugs being investigated as IND become a successful candidate for marketing, it should be noted that NDA number is a different number than an IND number. Once an NDA has been submitted, all correspondence for that NDA should reference that NDA number. FDA has 60 days to file that submitted NDA, or FDA could refuse to file an NDA due to format and content issues or absence of critical piece(s) of information/data needed for the FDA to make a decision on the approvability of the NDA.

Under the Prescription Drug User Fee Act of 1992 (PDUFA), the FDA has defined timeframes applicable to drug application reviews. The FDA usually takes 6 to 10 months from the date of submission of the NDA to make a decision of the acceptability of the application, often referred to as NDA action. This time frame depends on the type of NDA submitted. The FDA gives a priority designation for a product that if approved would be a significant improvement compared to marketed products in the treatment, diagnosis, or prevention of a disease. Evidence of increased effectiveness, elimination, or reduction of treatment related drug reactions, safety, and effectiveness in a new subpopulation, or enhanced patient compliance can demonstrate improvement. All applications not qualifying as priority are classified as standard applications. Priority applications are reviewed within six months, where as standard applications have a 10-month review clock. A decision regarding the assignment of a standard or a priority rating to the application is made before the 60 day filing of the NDA.

There are certain types of drug approval processes that facilitate the development and expedite the review of the new drugs that are intended to

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treat serious life threatening conditions and to demonstrate the potential as treatment for an unmet medical need. Some of these programs are the accelerated drug approval/fast track programs or rolling submissions. The accelerated drug approval program (Subpart H) is a highly specialized mechanism for speeding the development/review of drugs that promise significant benefit over existing therapy for serious or life-threatening illnesses like AIDS, cancer, Parkinson's disease etc., and for a condition for which no therapy exists. This program involves the modification of the criteria on which the approval is based on. It allows for approval to be based on a surrogate endpoint or an effect on a clinical end point other than survival or irreversible morbidity. Under such circumstances, the program may require appropriate post approval studies to validate the surrogate endpoint or otherwise confirm the effect on a valid clinical endpoint.

When certain sections of an application are accepted by the Agency prior to the receipt of the complete application, the submissions are referred to as rolling NDA submissions (i.e., pre-submission of pharm-tox reports, clinical study reports, and even data summaries and listings from the first of two or more pivotal trials). Sponsors of designated fast track products can request this type of submission by submitting certain completed portions of an NDA prior to submitting the other sections of the application. In such cases the sponsor is required to provide a schedule for submitting the information necessary to make the NDA submission complete. Further details of these programs can be found under Regulatory Guidance and Mapp (Manual of Policy and Procedure) on the FDA website [1, 3].

Sometimes there is a need for either an Advisory Committee Meeting or a face-to-face meeting with the sponsor to discuss issues that arise during the NDA review process. Once the NDA is submitted, pivotal study sites are identified and inspected for good clinical practices (GCP) and good laboratory practices (GLP) compliance by the Office of Compliance. An NDA action is taken after obtaining results from the inspection of the study site. The action could result in the approval or non-approval of an NDA, or in an approvable NDA. An approvable NDA implies that the information that has been reviewed by the FDA appears to be an acceptable data; however, some additional information is needed to approve the product for marketing in the United States. This could involve collection of additional data, data re-analysis or negotiation of labeling language. The overall NDA review process is shown schematically in Fig. 3.

Table 1 summarizes the type of studies that are typically part of the clinical pharmacology and biopharmaceutics plan for a new drug, and Table 2 gives an example of how all of the clinical pharmacology and biopharmaceutics information can be summarized concisely. Readers are

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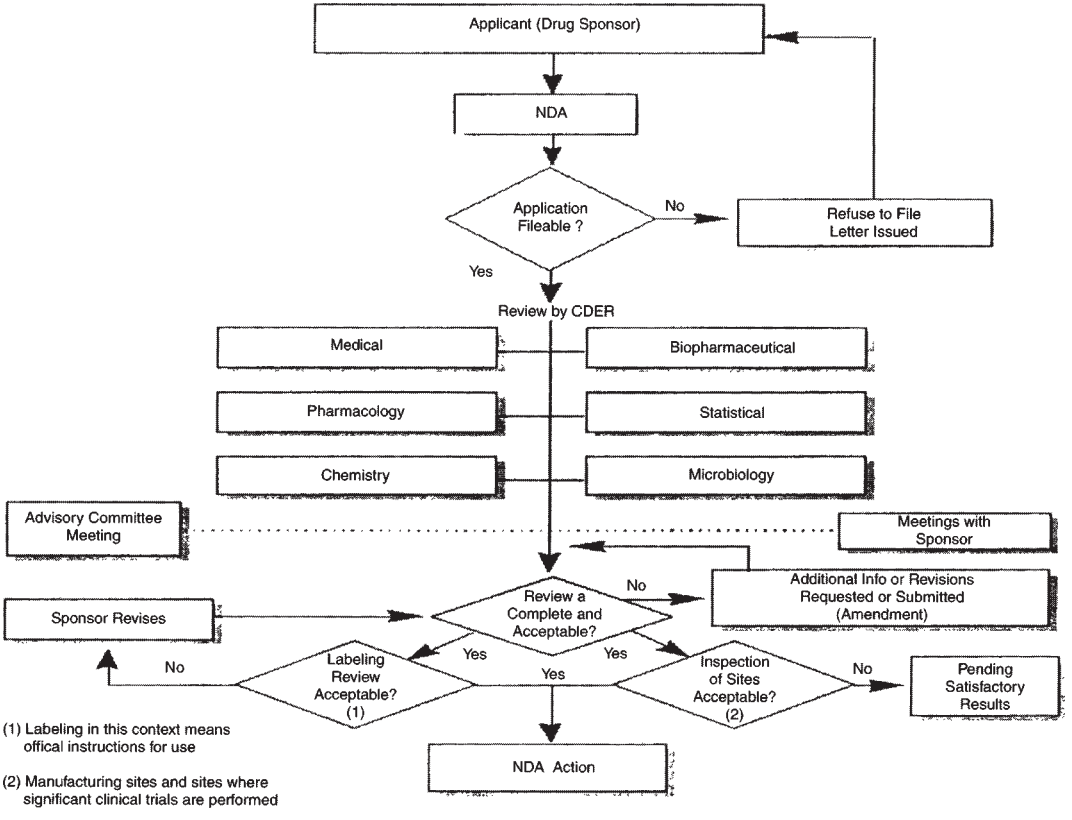

FIGURE 3 NDA review process, http://www.fda.gov/cder.

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TABLE 1 General list of Studies Submitted to Support the Clinical Pharmacology and Biopharmaceutics Portion of the NDA

Table of contents present and sufficient to locate reports, tables, data, etc.
Tabular listing of all human studies
HPK summary
Labeling
Reference bioanalytical and analytical methods
Bioavailability and bioequivalence studies
Mass balance study
BA studies
Absolute BA
Relative BA
BE studies
Average BE
Food–drug interaction
Dissolution tests (In vitro–in vivo comparison studies)
Studies using human biomaterials
Plasma protein binding studies
Blood/plasma ratio
Metabolism studies using hepatocytes, microsomes, etc.
Human pharmacokinetics studies
PK, and initial safety and tolerability in healthy
Volunteers
Single dose
Multiple dose
PK, and initial safety and tolerability in patient
Volunteers
Single dose
Multiple dose
Dose proportionality
Single dose
Multiple dose
PK in population subsets to evaluate effects of intrinsic factors
Ethnicity
Gender
Pediatrics
Geriatrics
Renal impairment
Hepatic impairment
PK to evaluate effects of extrinsic factors
Drug–drug interaction: Effects on primary drug
Population PK studies
Summary table of PK/PD studies
PK/PD studies in volunteers
PK/PD studies in patients
Individual data sets for all PK and
PK/PD studies in electronic format
Other
Genotype/phenotype studies
Chronopharmacokinetics

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also encouraged to refer to the FDA website and the ICH Common Technical Document that provides information on what information an new drug application should contain.

CLINICAL PHARMACOLOGY CONSIDERATIONS IN NEW DRUG DEVELOPMENT

In a new drug application, the OCPB reviewer is looking for data and analyses that provide a rational justification for the selected dose/dosing regimen as well as the sponsor's attempt to "individualize" doses in certain populations and/or scenarios, e.g., in pediatrics, in elderly, in renal/hepatic impairment, and in presence of concomitant medications. The sponsor usually generates this information in the IND stage of the regulatory process. The reader is also encouraged to read the article that describes the question-based review approach that the Office of Clinical Pharmacology and Biopharmaceutics follows [4]. The chapters presented in this book provide a general approach to drug development.

There may be some classes of drugs with certain characteristics (e.g., chirality), formulation (e.g., liposomes) or certain indications (e.g., biologicals) which may need additional consideration in their evaluation. Some of these cases are discussed in various chapters of this book.

BIOPHARMACEUTICS CONSIDERATIONS IN NEW DRUG DEVELOPMENT

Biopharmaceutics is a comprehensive term denoting the study of the influence of pharmaceutical formulation variables on the performance of the drug in vivo [5]. In a new drug application, the OCPB reviewer generally looks for the pH solubility profile, pKa of the drug substance, drug permeability or octanol/water partition coefficient measurement which may be useful in selecting the dissolution methodology and specifications.

Dissolution of the drug under physiological conditions is one of the factors assessing drug absorption after oral administration. Dissolution testing is required for all solid oral dosage forms in which absorption of the drug is necessary for the product to exert the desired therapeutic effect. In addition to predicting in vivo performance of the dosage units, dissolution tests help in assuring drug product quality from batch to batch and may also be a guide in the development of new formulations. The dissolution specifications set forth also ensure the drug product's sameness under scaleup and postapproval changes. Dissolution data also provides for assessing the waiver of a bioequivalence study. For NDAs the dissolution

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TABLE 2 Summary of Clinical Pharmacology and Biopharmaceutics Characteristic of the Drug

Drug Name: _____ Indication _____ Dosage Form _____

Dose: _____ Dosage Regimen_____

PK Characteristic	Dose (mg) in Healthy				Dose (mg) in Patients				Absolute *F*	Is Drug a Racemate	Dose Selection Criteria
	AUC	C_{max}	*CL*	*T1/2*	AUC	C_{max}	*CL*	*T1/2*			
Dose Proportionality	Dose (mg)				Dose (mg)				Dose (mg)	Dose (mg)	
	AUC	C_{max}	*CL*	*T1/2*	AUC	C_{max}	*CL*	*T1/2*	AUC C_{max} *CL* *T1/2*	AUC C_{max} *CL* *T1/2*	PK linear/nonlinear
Metabolism Mass Balance	Drug PK Dose (mg)				Radioactivity PK Dose (mg)						
	AUC	C_{max}	*CL*	*T1/2*	AUC	C_{max}	*CL*	*T1/2*	Mass Balance	Metabolites Identified	Activity of Metabolites
									% in Urine % in Feces % Total in *X* hours		
In vitro metabolism	Phase I Enzymes Involved				Phase II Enzymes Involved				Test Drug Inhibitor of	Test Drug Inducer of	Transporter
Pharmacogenomics Information											
Drug Interactions	Test Drug On				AUC	C_{max}	*CL*	*T1/2*	Other drugs on Test Drug		AUC C_{max} *CL* *T1/2*
	Drug *X* Drug *Y* Drug *Z*								Drug *X* Drug *Y* Drug *Z*		

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Special Population (Compared to Young Adult)	Parameters evaluated Compared to young Adults	Pediatric	Elderly	Gender	Race
	AUC C_{max} *CL* *T*1/2				

Special Population (Disease State)		Renal Impairment				Hepatic Impairment		
	Parameters	Mild	Moderate	Severe	Dialysis	Mild	Moderate	Severe
	AUC C_{max} *CL* *T*1/2							

Population PK and PK-PD	Covariates identified as responsible for PK variability	PK-PD Correlated to which surrogate marker, efficacy, adverse events	Does population PK and PK-PD agree with the dose selected by the sponsor (Y/N/not known)	Does the drug prolong QT interval Y/N

Dosage Adjustments Necessary for			Drug Interactions		
	Condition 1 Renal Impairment	Condition 2 Hepatic Impairment	Drug *X*	Drug *Y*	Drug Z
	Dose ↓ or ↑ by	Dose ↓ or ↑ by	Dose ↓ or ↑ by	Dose ↓ or ↑ by	Dose ↓ or ↑ by

Biopharmaceutics	Dissolution Method and Specs	BCS Class	Bio-waiver Request Y/N	BE of TBM dosage form	Other, e.g. IVIVC
				90% CI	

Phase IV Commitments

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specifications are based on acceptable clinical, pivotal bioavailability and/or bioequivalence batches.

Biopharmaceutics issues depend on the route of administration as well as the kind of dosage forms (oral versus other routes of administration, immediate release dosage form, and modified release dosage forms). Some of these issues have been covered in the various chapters of this book.

The final formulation the sponsor wishes to market may not always be the one that has been used during the drug development. These formulation changes may be necessary due to variety of reasons ranging from aesthetic to overall improvement in formulation performance or to accommodate manufacturing convenience. It is essential to know that the to-be-marketed formulation will perform in the same way as the clinical trial formulation performed in the pivotal clinical studies. For an NDA, bioequi valence studies provide a link between the pivotal and early clinical trial formulation, a link between the formulations used in the pivotal clinical trial, and the to-be-marketed formulation or any other comparisons as appropriate. Bioequivalence studies provide information on the product quality and performance, when there are changes in components, composition and method of manufacture after approval of the drug product. The FDA has provided Guidance for the industry, such as BA/BE guidance [6], SUPAC-IR [7], and SUP AC-MR [8], to determine when the changes in the components and composition and/or method of manufacture of the drug product suggest a need to perform further in vitro/in vivo studies. Although, SUP AC stands for Scale-up and Post Approval Changes to the formulation, the same principals outlined in these guidances are utilized at the preapproval stage of the drug to determine the level of data needed for bio waivers.

PRODUCT LABEL

One of the most important products of the drug development is the drug product labeling. Since this is the document that will be utilized by the prescribing Physicians to appropriately dose the patients, great care is taken by the FDA and Industry Scientist to provide accurate information in a clear and concise way in the product labeling. Labeling guides the prescriber, based on data obtained from clinical trials, in optimizing the dose and dosage regimen for all populations and outlines the adverse events which were experienced by patients in the clinical trials etc. Labeling generally has the following subheadings: Warnings, Description, Chemical Structure, Clinical Pharmacology, Indication and Usage, Contraindications, Precautions, Adverse Reactions, Overdosage, Dosage and Administration, How Supplied, and Product Photos. In general, Clinical Pharmacology

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sections describe the clinical studies conducted to obtain pharmacokinetic data in healthy subjects, patients, special populations and drug-drug interactions.

Precautions and contradictions will generally highlight data that would require a caution or adjustment of dose. The dosage administration section gives the approved dose and recommended dosage adjustments under special circumstances. Presently, there is an initiative where a working group at FDA is working on reforming the label so that important information for the prescriber is highlighted in the beginning of the label.

SUMMARY

Drug development is a complex process that requires collaboration of scientists with varying expertise. For any new drug being developed, teams of scientists are responsible within an industry to develop the drug, and a team of scientists at the FDA are responsible to review the IND and NDA submitted to the FDA.

Involvement of FDA scientists generally starts with the submission of a pre-IND meeting request by the sponsor. Although FDA scientists are involved and interact with the sponsor during the entire drug development process, some of the key interaction occurs when the sponsor submits an IND, drug development plan, pre-Phase 2 meetings, End-of-Phase 2 meeting, pre-NDA meeting, and when the protocols are submitted during the IND phase of development. For optimal drug development, FDA encourages sponsor to have open communication and reviewers are available to meet the industry scientists at any stage of drug development. These meetings provide a forum for interactive exchange of scientific ideas. To encourage and facilitate meeting between the industry and sponsor scientists, a document describing process of arranging meetings has been published as manual for policies and procedures for meetings and is published on the FDA website [1].

For ease of understanding and getting an overview of the drug development, it is important to summarize the assessment of new drug application in one table. One example of such a table has been provided in Table 2 in this chapter.

Once the FDA scientist has completed the review, the important part is to convey the data in a clear way, so that the physicians can make informed decision as to what is best for the patients. Readers are encouraged to look at completed NDA reviews available on FDAs, Freedom of Information (FOI) Website to gain insight into the regulatory issues that may arise during reviews of NDAs.

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In this chapter we have briefly covered the IND and NDA review process. However, it is beyond the scope of this book to cover in detail several regulatory considerations such as good clinical practices, good laboratory processes, advisory committee meetings, orphan drugs, supple-mental NDA, post approval changes, etc. Readers are referred to the FDA, ICH, and other regulatory agency Websites to get additional information or updates on scientific and regulatory issues related to new drug development.

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5

In-vitro Drug Metabolism Studies During Development of New Drugs

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INTRODUCTION

Since late 1980s, the drug discovery and development process has undergone significant changes, particularly in the preclinical stage involving drug candidate selection, drug metabolism and safety studies. These changes are directly related to the scientific progress in research areas of combinatorial chemistry, recombinant DNA technology, toxicology, metabolism, and analytical instrumentation. The increasing availability of tissues, cell cultures, and drug-metabolizing enzymes from human sources has led to the increased use of in vitro studies to select the most desirable drug candidates. Well executed in vitro studies can provide valuable information regarding the metabolic fate of a new drug in humans, critical

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factors contributing to the variability of pharmacokinetic parameters, and the potential for drug-drug interactions. Consequently, in vitro study results are now being routinely included in New Drug Applications (NDA) by the sponsors.

What type of in vitro studies should be included in the NDA? How should these studies be conducted? In this chapter, we describe some of the commonly used in vitro techniques used to study drug metabolism during drug development. However, as indicated in an FDA document on in vitro drug metabolism studies [1], the assessment of drug metabolism in vitro is a rapidly evolving area of drug development and regulation. Therefore, new methods and additional studies will undoubtedly be added to this list. Since one of the guiding principles in drug development is to generate data utilizing up-to-date scientific technology and knowledge available in the field, modification of currently used methods and approaches are expected with time. The goal of early in vitro studies conducted at the preclinical stage is to obtain optimal information to maximize the possibility of success in developing a safe and effective drug for clinical use.

METHODS TO ASSESS DRUG-DRUG INTERACTION POTENTIAL

In vitro studies are useful for assessing the potential of metabolism-based drug-drug interaction [2–4], a major concern for the effective and safe use of therapeutic agents and a critical factor contributing to the recent withdrawal of various drugs from the United States market [5–6]. Since cytochrome P450 plays a key role in the metabolism of numerous important drugs in clinical use, cytochrome P450-mediated drug-drug interactions have attracted most attention, although the importance of transporter-based drug-drug interactions has also been recognized in the last few years. Central to the issue of metabolism-based drug-drug interactions is the identification of the cytochrome P450(s) responsible for the metabolism of the interacting drugs. Major activity alterations of the involving cytochrome P450 species, due to either inhibition or induction, can result in potential, significant pharmacokinetic changes of interacting drugs in humans.

As described in the following sections, various in vitro methods can be used to assess the potential of drugs acting as inhibitors or inducers of cytochrome P450. If the potential for interaction is great, in vivo studies in human should be considered to evaluate the clinical significance of the in vitro findings. The in vivo approaches include specific pharmacokinetic and pharmacodynamic studies, population pharmacokinetic studies, and clinical safety and efficacy studies [7–9]. In vivo animal studies have limited values in predicting human drug-drug interactions, particularly if the results in animals are negative. A single change in amino acid of the protein

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sequence can dramatically change the substrate specificity of cytochrome P450 [10, 11]. In addition, various researchers have described species differences in cytochrome P450 inhibition [12, 14] and induction [13]. Thus, cytochrome P450s in the same gene family in animals and human may not respond to inhibitors and inducers in similar manners.

GENERAL APPROACHES

In vitro Methodologies

Most of the in vitro metabolism studies involve the use of tissues or drug-metabolizing enzymes from the liver. The emphasis of metabolic research has been on the liver, as it is considered the major organ for drug metabolism, and that we know the most about the properties and functions of liver drug-metabolizing enzymes, particularly cytochrome P450. In addition, human liver tissues and human recombinant cytochrome P450s are readily available. However, for some drugs, nonhepatic tissues, such as the gastrointestinal mucosa, may play a vital role in their metabolism. In these cases, in vitro metabolism studies employing tissues from the kidneys, intestines, or skin may be valuable. Similarly, although cytochrome P450s are the dominant enzymes for the metabolism of most drugs, other drug-metabolizing enzymes are also present in the liver and extrahepatic tissues. These non-cytochrome P450 enzymes are responsible for glucuronidation, sulfation, acetylation, glutathione conjugation, and other enzymatic reactions. In vitro studies using specific tissue fractions and cofactors are critical in characterizing these metabolic reactions. In this chapter, unless specifically indicated, all in vitro studies refer to cytochrome P450-mediated hepatic metabolism of new drugs.

Many in vitro models are available to study hepatic drug metabolism, ranging from the simplest recombinant enzymes to subcellular fractions, hepatocytes, liver slices, to the more complicated isolated, perfused liver. The degree of physiological relevance of these models decreases as one changes from the whole organ to the recombinant enzymes. It is important to select in vitro systems that are most suitable to achieve specific goals of the study [2]. If the hepatic subcellular fractions are to be used for metabolism studies, it is important to recognize the distribution of the enzymes responsible for the metabolic events in various tissues and the specific cofactors required for particular reactions.

One critical issue in conducting in vitro metabolism studies is the appropriateness of drug concentrations that are used in these studies. Since the drug concentration at the enzyme active site in the liver could not be

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easily measured and the plasma drug concentration is generally unknown at the time of in vitro metabolism study, it is often difficult to define the in vitro drug concentration of physiological relevance.

Despite this uncertainty, it is the general rule not to use unrealistically high drug concentrations (e.g., in the mM range) for in vitro metabolism studies. Considering the assay sensitivity and the general plasma drug concentrations in humans, drug concentrations in the low μM range represent a good range to study for most of the in vitro metabolism studies. A good practice is to use several drug concentrations (e.g., low, medium, and high, spanning two to three orders of magnitudes) in these studies. This is desirable particularly for drugs that undergo metabolism via two or more pathways involving multiple enzymes (with different Km values). In this case, both high and low affinity metabolic pathways can be studied.

With the advancement in analytical methodologies and knowledge of human drug-metabolizing enzymes, the major metabolic pathways of a new drug in humans can be readily established and metabolites can be isolated from in vitro models. If the metabolites are found to be pharmacologically active, sensitive and specific assays could be developed to assess the pharmacokinetic profile of the metabolite(s) in subsequent clinical studies.

Animal toxicity studies are an important component of safety evaluation of new drugs. Comparative animal and human metabolic profiles generated in vitro can help the selection of appropriate animal models for toxicity evaluation and may be useful in the interpretation or hypothesis-generating of certain clinical findings.

The liver slices and hepatocyte suspensions from human and animal species are suitable for metabolic profiling, since these systems contain all the necessary enzymes and cofactors for metabolism [2]. Hepatic subcellular fractions and recombinant drug-metabolizing enzymes can be used when metabolic profiles are relatively simple and only one or two well-recognized enzymes are involved in the biotransformation of the new drug. Because of the known genetic polymorphism of many of the human drug-metabolizing enzymes and the well-recognized large inter-individual variability in drug metabolism, it is desirable to use liver tissues derived from more than one individual (if possible) to generate metabolic profiles. In addition, as fresh human livers are not always readily available, cryopreserved human hepatocytes are now being increasingly used for drug metabolism studies [3]. Cryopreserved human hepatocytes retain most, if not all, of the major drug-metabolizing enzyme activities.

In vitro/In vivo Correlation

Although significant progress has been made in recent years in the evaluation of drug-drug interaction potential based on in vitro data, a

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complete understanding of the relationship between in vitro findings and in vivo human results of metabolism-based drug-drug interaction studies is still emerging. In some cases, excellent correlation of in vitro and in vivo results has been demonstrated while in others, the in vitro and in vivo correlation has been poor [15]. Because of the complexities of various factors impacting both in vitro and in vivo drug-drug interactions, accurate predictions of the extent of in vivo drug interactions from in vitro metabolic studies will require continued efforts in obtaining additional high quality correlation data to permit rational evaluation of new drugs. At the present time, the feasibility of predicting in vivo drug interactions based on in vitro metabolic data is still under rigorous debate. Some investigators believe that a quantitative prediction of in vivo drug interaction is possible [16–18] while others take the position that a qualitative prediction approach is more feasible [19, 20]. In a recent commentary, Tucker et al. [21] used the qualitative terms "low risk, medium risk, and high risk" to describe the projection of AUC changes based on the [I]/Ki ratio, where the Ki values are determined from in vitro studies.

Various factors contributing to the difficulty in predicting if a new molecular entity (NME) is an inhibitor from in vitro data. Among them, the unusual cytochrome P450 property and the large number of drug substrates appear to be critical factors. In vitro drug-drug interaction patterns (e.g., mutual inhibition, partial inhibition, activation, and lack of reciprocal inhibition) for a given cytochrome P450, such as CYP3A4, are often substrate-dependent. The Ki value of an inhibitor for a given cytochrome P450 is dependent on the probe substrates, enzyme sources, and experimental conditions such as protein concentration and incubation time due to various degrees of inhibitor-protein binding, partition of inhibitor to the lipid and aqueous layers, and inhibitor and substrate depletion.

One of the challenges in predicting the extent of in vivo drug-drug interaction from in vitro metabolism studies is the lack of information on the inhibitor concentration in vivo in the active site of the enzyme or tissues. Since the plasma inhibitor concentration may be the only known parameter, both total inhibitor concentration and unbound inhibitor concentration have been used for in vitro-in vivo correlation evaluation. Claims of good correlation with either of the parameters have been reported for different drugs. Other factors contributing to the lack of good in vitro-in vivo correlation using either of the parameters may include the following: (1) the inhibiting drug may also act as an inducer; (2) other parallel elimination pathways and/or extrahepatic metabolism of the drug may decrease the importance of the in vitro-assessed pathway; (3) modulation of an important cellular transport mechanism by the inhibitor may change the extent of in vivo drug-drug interaction, and (4) rapid elimination of

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inhibitor in vivo by noncytochrome P450 pathways may decrease the extent of in vivo drug-drug interaction.

Study Design Considerations

Cytochrome P450 Identification

Unequivocal identification of one or more specific cytochrome P450 enzymes responsible for the metabolism of new therapeutic agents is the cornerstone of in vitro metabolism studies. This information is also critical for the follow-up cytochrome P450 inhibition and induction studies in the overall evaluation of in vitro drug-drug interactions. For all these studies, the experimental conditions should be that the measured initial reaction rates (in terms of product formation) are linear with respect to enzyme concentration and incubation time. It is preferable to use low enzyme concentration (e.g., below 0.5mg human liver microsomal protein per mL) and short incubation time (less than 20 min) to minimize protein binding and depletion of substrate and inhibitor (no more than 20% consumption, preferably less than 10%). If the analytical sensitivity is not an issue, lower enzyme concentration and shorter incubation time are highly desirable. In case of a slow substrate turnover, higher enzyme concentration and longer incubation time can be used as long as the initial metabolic rates are being measured.

If the cytochrome P450-mediated metabolism represents a significant clearance mechanism for the NME, cytochrome P450 reaction phenotyping should be carried out, generally, with human liver microsomes and recombinant cytochrome P450s using a combination of several basic approaches [22]. The NME concentrations used are generally at or below the Km values. Initial reaction rates are measured in the absence and the presence of antibodies or chemical inhibitors, or with a panel of human liver microsomes for correlation analysis with various cytochrome P450 probe substrates. If there is an indication for the involvement of more than one cytochrome P450 in the metabolism of the drug, several drug concentrations (e.g., low, medium, and high-spanning two to three orders of magnitude) should be used for inhibition studies.

Chemical Inhibitors and Inhibitory Antibodies. Specific and potent inhibitors are valuable for cytochrome P450 reaction phenotyping. In this respect, inhibitory antibodies (particularly monoclonal antibodies) with demonstrated specificity and potency can be useful [23], as illustrated in a recent paper by Granvil et al. [24]. These investigators described that the 4-hydroxylation of debrisoquine, a well-recognized probe reaction of CYP2D6, is mediated not only by CPY2D6 but also by human CYP1A1. Whereas quinidine, a recognized selective inhibitor of CYP2D6, inhibits the

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4-hydroxylation of debrisoquine by both CYP2D6 and human CYP1A1, anti-CYP2D6 monoclonal anitbody inhibits specifically CYP2D6-medicated reaction, and not CYP1A1-dependent metabolism. To date, specific and potent monoclonal as well as polyclonal antibodies have not been widely used by the pharmaceutical industry possibly due to their high cost and limited availability from commercial sources.

A desirable antibody inhibition study can be conducted in two stages. Initially, metabolism of a drug by pooled human liver microsomes is examined in the presence of antibodies against all major human cytochrome P450s at a single high concentration (known to give greater than 80–95% inhibition with probe substrates) to determine which antibodies significantly inhibit the metabolism. This study establishes that one or more cytochrome P450 is involved in the metabolism of an NME. In subsequent studies, the effect of those inhibitory antibodies on the metabolism of the NME is studied in more detail using a series of antibody concentrations. A well-designed study should show that metabolism is inhibited strongly by the specific antibody in a concentration-dependent manner at low antibody concentrations and then reaches maximum inhibition at higher antibody concentrations [25] as illustrated in Fig. 1 (curves A and D). A steep inhibition slope indicates high potency of the antibody against specific cytochrome P450. The extent of the maximum inhibition indicates the extent (%) of the metabolism of the NME by this particular cytochrome P450 enzyme. No meaningful conclusion can be made regarding the role of a specific cytochrome P450 in the metabolism of an NME when an antibody inhibition study showed a shallow inhibition slope (an indication of low antibody potency) and failed to demonstrate maximum inhibition (Fig. 1, curve B). Thus, a good antibody inhibition study establishes not only the involvement but also the quantitative importance of a particular cytochrome P450 in the metabolism of the NME. When it is desirable to obtain information regarding the variability of cytochrome P450 involvement, particularly when more than one cytochrome P450 enzymes are involved, similar studies can be carried out with a panel of human liver microsomal preparations. Frequently, one can demonstrate a wide range of involvement of specific cytochrome P450 in the metabolism of a particular drug with microsomes from different donors [23].

Although specific chemical inhibitors for individual human cytochrome P450 are rare, isoform-selective inhibitors are generally available at most pharmaceutical laboratories and are valuable when properly used. Table 1 lists preferred probe substrates and inhibitors for individual cytochrome P450 enzyme [21]. Similar to antibody inhibition studies, chemical inhibition studies can be carried out first with a single inhibitor concentration (known to give strong inhibition with probe substrates) to

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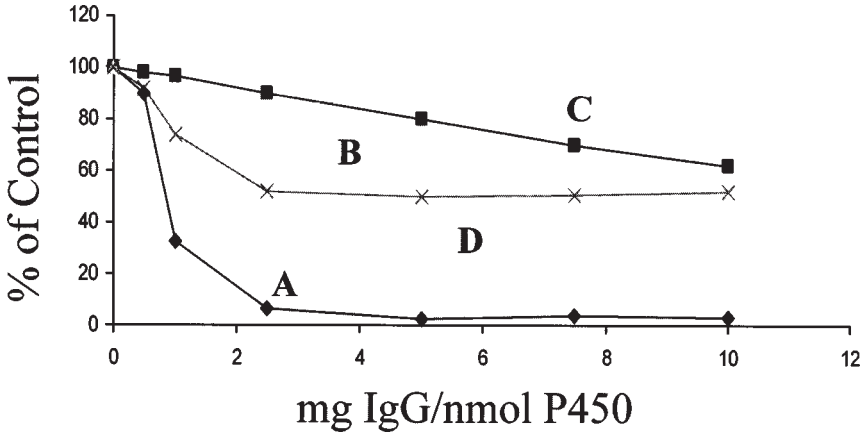

FIGURE 1 Inhibition of human liver microsomal drug metabolism by antibodies against cytochrome P450. *Curve A* depicts the strong inhibition of compound A metabolism by anti-CYP3A4 antibodies. The steep inhibition slope at low antibody concentrations indicates high potency of this antibody preparation. Maximum inhibition at higher antibody concentrations indicates that greater than 90% of the metabolism of compound A is mediated by CYP3A4 in this pooled human liver microsomal sample. *Curve B* shows the inhibition of compound A metabolism in human liver microsome by a different anti-CYP3A4 antibody preparation. The shallow inhibition slope indicates that either this antibody has a low potency against CYP3A4 or it cross-reacts with another cytochrome P450. No conclusion can be made regarding the role of CYP3A4 in the metabolism of compound A. *Curve C* is the control experiment showing lack of inhibition of compound A metabolism by pre-immune IgG. *Curve D* depicts the inhibition of the metabolism of compound B by anti-CYP3A4 antibodies. The steep inhibition slope is noted at low concentrations of this potent antibody. CYP3A4 is responsible for 50% of the

determine which probe inhibitors significantly inhibit the metabolism of the NME, followed by a more detailed study involving a series of concentrations of the inhibitors. As shown in Fig. 2 (curves A and B), a good chemical inhibitor selective for a given cytochrome P450 isoform should give strong inhibition (a steep inhibition slope) in the metabolism of an NME at low inhibitor concentrations and reach maximum inhibition at higher inhibitor concentrations so that the quantitative involvement of this cytochrome P450 isoform in metabolism can be established. Gradual increase in inhibition with a wide range of inhibitor concentrations (i.e., a shallow inhibition slope, Fig. 2, curve C) would suggest that the inhibitor either has low potency toward the particular cytochrome P450 or it acts as a poor substrate of the enzyme. In this case inhibition results from the study have limited values. When studies are carried out using a panel of human

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TABLE 1 Recommended in vitro Probe Substrates and Inhibitors for CYPs (Ref. [21])

	Substrates		Inhibitors	
CYP	"Preferred"	"Acceptable"	"Preferred"	"Acceptable"
1A2	Ethoxyresorufin Phenacetin	Caffeine (low turnover) Theophylline (low turnover) Acetanilide (mostly applied in hepatocytes) Methoxyresorufin	Furafylline	α-naphthoflavone (but can also activate and inhibit CYP3A4)
2A6	Coumarin			Coumarin (but high turnover)
2B6	S-Mephenytoin (N-desmethyl metabolite)	Bupropion (availability of metabolite standards?)		Sertraline (but also inhibits CYP2D6)
2C8	Paclitaxel (availability of standards?)		("glitazones"—availability of standards?)	
2C9	S-Warfarin Diclofenac	Tolbutamide (low turnover)	Sulphaphenazole	
2C19	S-Mephenytoin (4-hydroxy metabolite) Omeprazole			Ticlopidine (but also inhibits CYP2D6) Nootkatone (but also inhibits CYP2A6)
2D6	Bufuralol Dextromethorphan	Metoprolol Debrisoquine Codeine (all with no problems, but less commonly used)	Quinidine	
2E1	Chlorzoxazone	4-nitrophenol Lauric acid		4-methyl pyrazole
3A4	Midazolam Testosterone (strongly recommended to use at least two structurally unrelated substrates)	Nifedipine Felodipine Cyclosporin Terfenadine Erythromycin Simvastatin	Ketoconazole (but recent evidence indicates that it is also a potent inhibitor of CYP2C8) Troleandomycin	Cyclosporin

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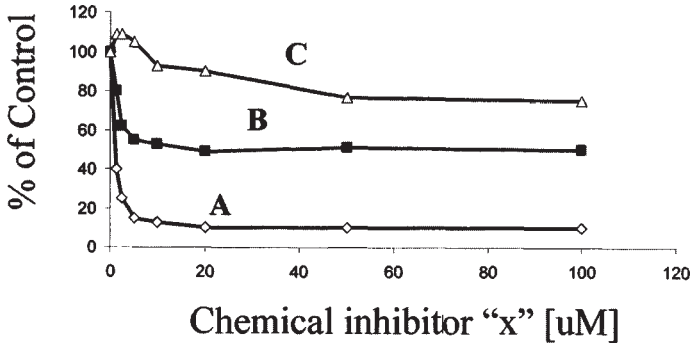

FIGURE 2 Inhibition of human liver microsomal drug metabolism by a chemical inhibitor of CYP3A4. *Curve A* depicts the strong inhibition of compound A metabolism by this inhibitor. The steep inhibition slope at low inhibitor concentrations indicates that this inhibitor of CYP3A4 is very potent. CYP3A4 contributes to approximately 90% of the metabolism of compound A in this pooled microsomal preparation. *Curve B* shows that CYP3A4 contributes to 50% of the microsomal metabolism of compound B. *Curve C* depicts the shallow inhibition slope indicating poor inhibition of the metabolism of compound C even at high inhibitor concentrations. No conclusions can be made regarding the role of CYP3A4 in the metabolism of compound C.

liver microsomal preparations, different degrees of maximum inhibition in metabolism provide information regarding the variability of specific cytochrome P450 involvement in the metabolism of the NME among individual subjects.

Recombinant Human Cytochrome P450 Enzymes. Microsomes containing individually expressed human cytochrome P450s provide a different approach for cytochrome P450 reaction phenotyping. This approach establishes the intrinsic capability of the individual cytochrome P450 in the metabolism of an NME, in the absence of other cytochrome P450 species. If one or more cytochrome P450 species are involved in an NME's metabolism, it is important to examine the contribution of each cytochrome P450 to human liver microsomal metabolism using inhibitory antibodies or chemical inhibitors. Sometimes, a recombinant cytochrome P450 found to be involved in an NME's metabolism, based on a recombinant enzyme study, may later be shown to play little or no role in liver microsomal metabolism of the drug in the presence of other cytochrome P450s, based on an inhibition study. Furthermore, for these cytochrome enzymes for which activities are observed initially, a determination of the enzyme kinetics (Km and Vmax) may be warranted so that the intrinsic clearance and the relative importance of these different

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cytochrome P450 species contributing to the metabolism of the NME can be evaluated [26–28].

Correlation Analysis. Using this approach, the drug is incubated with a panel of human liver microsomes (preferably more than 10 preparations) and the reaction rates of an NME determined in each preparation are correlated with the reaction rates of a cytochrome P450 probe substrate measured in the same microsomal preparation. If a particular cytochrome P450 is responsible for the metabolism of the NME, a high correlation should be observed between the metabolic rates of the drug and the marker substrate. However, this type of correlation analysis appears to be less reliable in identifying specific cytochrome P450 enzymes responsible for the metabolism of an NME. For example, Weaver et al. [29] reported that 58C80 hydroxylation is catalyzed by CYP2C9 based on inhibition and recombinant cytochrome P450 studies; however, there is no correlation between 58C80 hydroxylation and CYP2C9 probe substrate activity (r=0.023). In another study, Heyn et al. [30] reported that although high correlations between S-mephenytoin N-demethylation and CYP2B6 (r=0.91), CYP2A6 (r=0.88), and CYP3A4 (r=0.74) were observed, other approaches showed CYP2B6 to be the major enzyme responsible for S-mephenytoin N-demethylation while CYP2A6 and CYP3A4 played no significant role in this reaction.

Cytochrome P450 Inhibition

It is important to examine if an NME is an inhibitor of cytochrome P450s not involved in the metabolism of the drug. For this type of study, the effect of NME on the metabolism of probe substrate for each of the individual cytochrome P450 (see Table 1) is evaluated, usually in human liver microsomes, although individual recombinant human cytochrome P450 enzymes have also been used. The incubation conditions should be such that initial rates could be measured. To determine the Ki value for any specific cytochrome P450, at least four to five probe substrate concentrations and two to three NME concentrations should be used in the assays. Substrate concentrations should cover a wide range (preferably 10–20-fold) with the number of concentrations evenly distributed below and above the Km value. The importance of proper selection of both substrate and inhibitor concentrations in these studies is well illustrated in the paper by Madan et al. [22]. The rates of metabolite formation of probe substrate are determined in the presence and absence of the NME inhibitor and the data are displayed in graphical representation to determine Ki and the type of inhibition [22]. Substrate-dependent inhibition has been reported earlier for CYP3A [49, 51]. Two or more substrates may be needed when evaluating inhibitors of CYP3A using in vitro methods [21, 47, 49]. Because of

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significant solvent effects (particularly when concentration >1%) reported for various CYP enzyme studies, low solvent concentrations should be used in these in vitro studies [47].

In addition to reversible inhibition, time-dependent inhibition of cytochrome P450 activity by a drug candidate may also be examined to determine if the NME is a mechanism-based inhibitor. For this type of study, an NME, at various concentrations (covering a 10–20-fold range), is preincubated with human liver microsomes with and without NADPH for various lengths of time (e.g., 0, 10, 20, 30, 45, and 60min) to allow the generation of reactive metabolites that inhibit cytochrome P450 activity irreversibly or quasi-irreversibly [22]. At various incubation time points, an aliquot of the samples is removed and diluted several folds with fresh assay buffer. The activity of the remaining cytochrome P450 is determined by the reaction rates of a probe substrate, and the data are displayed in graphical representation to determine the Ki and Kinact values [22, 31].

If an NME and clinically co-administered drugs are metabolized by the same cytochrome P450 isoform, inhibition of this cytochrome P450 can lead to the accumulation of either of the drugs and thereby cause potential serious drug-drug interactions. This potential can be evaluated using an in vitro system of human liver microsomes in the presence of both the drugs. The importance in the proper use of concentrations of either of the drugs is as described in the preceding section. The Ki value for either of the drugs can be determined and the potential of drug-drug interaction of co administered drugs can be evaluated.

Cytochrome P450 Induction

Cytochrome P450 induction represents another mechanism for metabolismbased drug-drug interactions, although it is much less common than inhibition-mediated interaction events. Drug treatment can result in the induction of cytochrome P450 responsible for its own metabolism (i.e., auto-induction) or other cytochrome P450s responsible for the metabolism of co-administered drugs. The major effect of cytochrome P450 induction is the alteration of drug efficacy and safety over time due to increased clearance of therapeutic agents resulting in decreased parent drug concentrations and increased metabolite levels.

To determine if an NME is a cytochrome P450 inducer, the compound, at several concentrations, is incubated with primary human hepatocytes for two to five days, and the metabolic rates for probe substrates of individual cytochrome P450 (generally CYP1A2, 2C9, 2C19, and 3A) are measured [32, 33]. The NME concentrations should be relevant to its therapeutic range or, if the theoretical range is not known, a pilot study covering two to three orders of magnitude may be appropriate. The enzyme activity is

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considered to be the most relevant measure while mRNA and Western blot analyses are useful primarily for mechanistic interpretation [21, 50]. In view of the individual variability in cytochrome P450 induction, primary human hepatocytes prepared from at least three individual donor livers should be used to obtain reliable results. Appropriate positive controls (e.g., omeprazole for CYP1A2 induction, rifampicin for 2C9, 2C19, and 3A4 induction) should be included in the study.

In addition to primary human hepatocytes, other in vitro methods such as receptor ligand assay and reporter gene assay have also been used to evaluate the intrinsic induction potential of drug candidates [13, 32, 34]. A positive result of the in vitro induction study can help design clinical trials to determine if induction is likely to occur at clinical doses and if the extent of induction may result in significant drug-drug interactions.

Transferases

If an NME is primarily metabolized by a noncytochrome P450 enzyme, it may become necessary to identify the specific enzyme form responsible for the metabolism of the compound, particularly if a co-administered drug is also biotransformed by a similar metabolic pathway and the same enzyme. However, for enzymes such as flavin-containing monooxygenases, monoamine oxidases, epoxide hydrolases, glucuronosyl transferases (UGT), sulfotransferases, methyltransferases, acetyltransferases, and glutathione-S-transferases, analytical tools are generally not available for carrying out reaction phenotyping experiments. For example, specific or highly selective probe substrates and inhibitors are still not available for most of these enzymes. In addition, antibodies against many of these enzymes are often noninhibitory so that antibody inhibition experiments can not be performed to identify the specific enzyme form(s) involved in the metabolism of an NME. For some of the enzymes, recombinant isoforms remain the only tool for reaction phenotyping.

When a drug molecule contains functional groups such as—OH,—NH2,—SH or—COOH, glucuronidation often represents the most important pathway for its clearance. Therefore, considerable attention has been paid to UGT reaction phenotyping and its role in drug-drug interactions [35, 39]. At the present time, highly selective chemical inhibitors and inhibitory antibodies for individual UGT isoforms are not available. The only method available to identify the specific isoform responsible for the metabolism of a drug is to conduct a study with recombinant UGT enzymes. In addition, a study using a combination of drugs in human liver microsomes or recombinant system may be valuable in order to determine if one drug inhibits the metabolism of the other drug or if mutual inhibition occurs.

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In the literature, there are limited clinical data on UGT-dependent drug-drug interaction [35], either because of the generally high Km and Ki for UGTs (therefore low intrinsic clearance and low interaction potential) or due to the lack of clinical studies designed to address UGT-dependent drug-drug interactions. Further studies are needed to evaluate the clinical significance of UGT-dependent drug-drug interactions.

Transporters

It has become increasingly evident that drug transporters, such as P-glycoprotein, play an important role in the absorption, distribution, and excretion of many drugs [36–38, 40]. Many substrates, inhibitors, and inducers of CYP3A4 are also substrates, inhibitors, and inducers of P-gp [40–45]. Drug-Drug interactions involving transporters, particularly P-glycoprotein, have become the new focuses in drug discovery and development. When drugs compete for the same binding sites on the P-glycoprotein molecule, drug-drug interactions can occur.

To determine if an NME is a substrate of P-glycoprotein and whether the compound acts as an inhibitor of P-glycoprotein, various in vitro systems, such as Caco-2 cells, cDNA-transfected Madine-Darby canine kidney cells and LLC-PK1 pig kidney cells, and derivative cells containing MDR1 (L-MDR1) can be used. Many studies use digoxin and vinblastine as in vitro probes and fexofenadine and digoxin as in vivo probe substrates of P-glycoprotein. The experiments are usually carried out under linear condition, and the substrate concentrations are at or below their Km values. Although ATPase and calcein-AM assays have been used, it appears that the efflux assay (also known as the bi-directional permeability assay) is the method of choice for evaluating compounds [38, 41].

At the present time, the in vitro methodologies have not been standardized for the identification of substrates and inhibitors for P-glycoprotein and other transporters. Prediction of the in vivo drug-drug interactions from in vitro studies is still problematic. It is expected that more selective probe substrates and inhibitors will be available for P-glycoprotein and other transporters (e.g., OATP, MRP, BCRP) in the future, and that our ability to predict drug-drug interactions in vivo at the transporters level will be greatly improved.

REGULATORY CONSIDERATIONS

Evaluation of an NMEs drug-drug interaction potential is an integral part of the regulatory review prior to its market approval [1, 7]. The clinical pharmacology and biopharmaceutic review of an NDA focuses on key questions relevant to the review and integrates information across various

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studies [46]. For example, in addition to questions addressing how the following intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) may influence exposure and/or response, the reviewers also ask questions related to extrinsic factors:

- What extrinsic factors (co-administered drugs, herbal products, diet, smoking, and alcohol use) influence exposure and/or response and what is the impact of differences, if any, in exposure on pharmacodynamics of an NME?
- Based upon what is known about exposure-response relationships and their variability, what dosage regimen adjustments, if any, do you recommend for each of these factors?

Among drug-drug interaction questions, the following may be addressed via in vitro studies:

- Is there an in vitro basis to suspect in vivo drug-drug interaction?
- Is the drug a substrate of CYP enzymes?
- Is the drug an inhibitor and/or an inducer of CYP enzymes?
- Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?
- Are there other metabolic/transporter pathways that may be important?

Depending on the answers to the above questions, additional studies may be conducted to fully assess the interaction potential of an NME with other drugs, herbal products, and/or food/juices. Figure 3 illustrates one algorithm in the evaluation of CYP enzyme-based drug-drug interactions of an NME; starting with in vitro evaluations of the metabolic profile and the CYP enzyme-modulating effects of the NME using human enzymes. Based on the outcomes of these in vitro evaluations, which are reviewed along with additional in vivo clearance information, further clinical studies may be conducted (Fig. 3).

The appropriate use of in vitro metabolism and drug interaction information can provide the basis for the design of subsequent in vivo studies, or obviate the need for further in vivo studies, as illustrated in the following two cases. For example, Drug A's effects on various cytochrome P450 enzyme activities have been evaluated with the following probe reactions (phenacetin O-deethylation for CYP1A2; tolbutamide 4'-hydroxylation for CYP2C9, S-mephenytoin 4'-hydroxylation for CYP2C19, bufuralol 1'-hydroxylation for CYP2D6 and testosterone 6β-hydroxylation for CYP3A) using human liver microsomes. The data show

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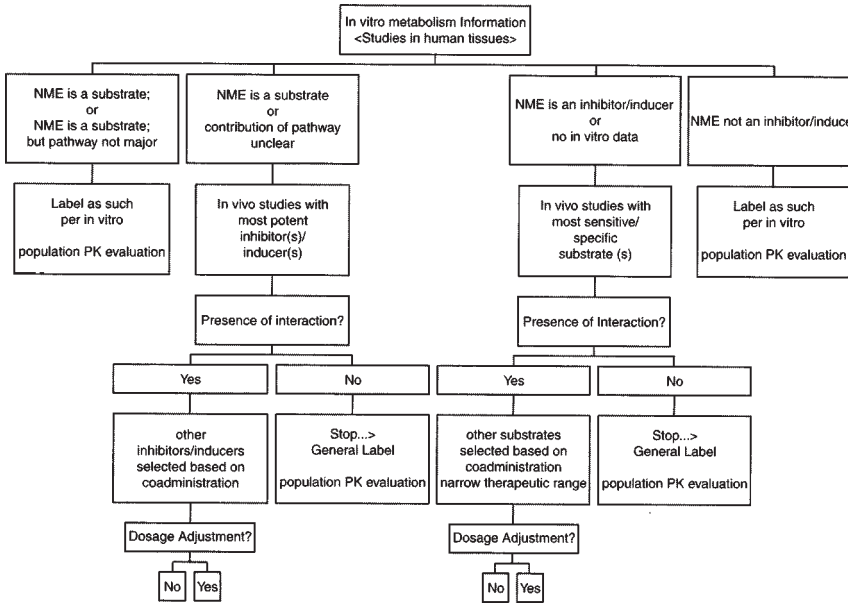

FIGURE 3 An algorithm for evaluating drug-drug interactions [21].

that Drug A does not inhibit CYP1A2, CYP2C9, CYP2C19, and CYP2D6 at concentrations 100-fold the mean steady state Cmax level achievable after the administration of the highest proposed clinical dose. Based on this information, no further in vivo studies on Drug A's inhibitory effects on CYP1A2, 2D6, 2C9, and 2C19 will be needed. Drug A inhibits CYP3A. Further analysis indicates the Ki value to be 1/100 of the Cmax level; suggesting Drug A to be a strong CYP3A inhibitor. A follow-up clinical study with oral midazolam administration confirmed its effect on substrates of CYP3A. The focus of the clinical evaluation on CYP3A has provided data useful for risk/benefit evaluation of Drug A and subsequent product labeling. Similarly, Drug B has been evaluated using in vitro methods and shown to have Ki values in the following rank order: CYP1A2=CYP2C9>CYP3A>CYP2C19>CYP2D6. As many of these I/Ki ratios fall within the gray area between "low risk" and "high risk" (21), an in vivo study focused on CYP2D6 was performed. By focusing on the CYP enzyme that appeared to be affected most by Drug B, the lack of interaction from this latter in vivo study would eliminate the need to study Drug B's effects on the other CYP enzymes.

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LABELING

In a proposed revision of physician labeling format and content, significant (or evidence of no) drug-drug interactions would appear in the Highlights section, in addition to having this information in the main body of the labeling [48]. In vitro and in vivo information on the metabolic pathways and metabolites, including contribution of specific enzymes, and known or expected effects of inducers or inhibitors of the pathway, is described in the *clinical pharmacology* section of the labeling. Any information on pathways or interactions that have been ruled out by in vitro data is also included in this section. Important clinical consequences of this information would be placed in *drug interactions, warnings, precautions, boxed warning, contraindications,* and *dosage and administration* sections of the main labeling, as appropriate. Examples of appropriate labeling language are provided in italic below:

> [Case 1] *In vitro* interaction has been studied for the new drug and no interactions have been demonstrated; no *in vivo* studies have been conducted to confirm or refute the *in vitro* finding.
>
> In vitro *drug interaction studies reveal no inhibition of the metabolism of the new drug by the CYP3A4 inhibitor ketoconazole. No clinical studies have been performed to evaluate this finding. However, based on the* in vitro *findings, a metabolic interaction with ketoconazole, itraconazole, and other CYP3A4 inhibitors is not anticipated.*

Recent examples, such as rosiglitazone (inhibitory effect on CYP enzymes), and sildenafil (inhibitory effects on CYP1A2, 2C9, 2C19, 2D6, 2E1, and 3A4), are listed in Table 2.

> [Case 2] Through *in vitro* investigations, specific enzymes have been identified as metabolizing the test drug, but no *in vivo* or *in vitro* drug interaction studies have been conducted.
>
> In vitro *drug metabolism studies reveal that the new drug is a substrate of the CYP ____ enzyme. No* in vitro *or clinical drug interaction studies have been performed. However, based on the in vitro data, blood concentrations of the new drug are expected to increase in the presence of inhibitors of the CYP ____ enzyme such as _____, _____, or.*

Recent examples, such as pimozide (substrate of CYP3A, ventricular arrhythmia observed in patients also taking CYP3A inhibitors, macrolide antibiotics) and Ketoconazole are listed in Table 2.

Recently approved product labels have reflected the increased understanding of metabolic pathways and consequences of drug

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TABLE 2 Labeling Examples of Metabolism and Drug-Drug Interaction Information

Drug name (brand name)	Labeling section	Labeling statement
Rosiglitazone (AVANDIA)	CLINICAL PHARMACOLOGY	*Metabolism* In vitro data demonstrate that rosiglitazone is predominantly metabolized by Cytochrome P_{450} (CYP) isoenzyme 2C8, with CYP2C9 contributing as a minor pathway.
	DRUG INTERACTIONS	*Drugs metabolized by cytochrome P_{450}* In vitro drug metabolism studies suggest that rosiglitazone does not inhibit any of the major P_{450} enzymes at clinically relevant concentrations. In vitro data demonstrate that rosiglitazone is predominantly metabolized by CYP2C8, and to a lesser extent, 2C9.
VIAGRA (sildenafil)	DRUG INTERACTIONS	*Effects of other drugs on VIAGRA* In vitro studies: Sildenafil metabolism is principally mediated by the cytochrome P450 (CYP) isoforms 3A4 (major route) and 2C9 (minor route). Therefore, inhibitors of these isoenzymes may reduce sildenafil clearance. *Effects of VIAGRA on other drugs* In vitro studies: Sildenafil is a weak inhibitor of the cytochrome P450 isoforms 1A2, 2C9, 2C19, 2D6, 2E1, and 3A4 (IC50 $>$ 150 μM). Given sildenafil peak plasma concentrations of approximately 1 μM after recommended doses, it is unlikely that VIAGRA will alter the clearance of substrates of these isoenzymes.
ORAP (pimozide)	CLINICAL PHARMACOLOGY	*Metabolism and pharmacokinetics* Pimozide is extensively metabolized, primarily by N-dealkylation in the liver. This metabolism is catalyzed mainly by the cytochrome P450 3A (CYP 3A) enzymatic system and to a lesser extent by cytochrome P450 1A2 (CYP 1A2).
	CONTRA-INDICATIONS	Ventricular arrhythmias have been rarely associated with the use of macrolide antibiotics in patients with prolonged QT intervals, as might be produced by ORAP. Specifically, two sudden deaths have been reported when clarithromycin was added to ongoing pimozide therapy. Furthermore, some evidence suggests that pimozide is metabolized partly by the enzyme system cytochrome P450 3A (CYP 3A). Macrolide antibiotics are inhibitors of CYP 3A, and thus could potentially impede pimozide metabolism. For these reasons, ORAP is contraindicated in patients receiving the macrolide antibiotics clarithromycin, erythromycin, azithromycin, dirithromycin, and troleandomycin.

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TABLE 2 Continued.

Drug name (brand name)	Labeling section	Labeling statement
		Because azole antifungal agents are also inhibitors of the CYP 3A enzymes and thus may likewise impair pimozide metabolism, ORAP is contraindicated in patients receiving the azole antifungal agents itraconazole and ketoconazole. Similarly, protease inhibitor drugs are also inhibitors of CYP 3A, and thus ORAP is contraindicated in patients receiving protease inhibitors such as ritonavir, saquinavir, indinavir, and nelfinavir. (See *PRECAUTIONS—Drug Interactions*.)
		Nefazone is a potent inhibitor of CYP 3A, and its concomitant use with ORAP is also contraindicated.
		Other drugs that are relatively less potent inhibitors of CYP 3A should also be avoided, in view of the risks, e.g., zileuton.
NIZORAL (ketoconazole)	CONTRA-INDICATIONS	Coadministration of terfenadine or astemizole with ketoconazole tablets is contraindicated. (See *BOX WARNING*, *WARNINGS* and *PRECAUTIONS* sections.)
		Concomitant administration of NIZORAL® Tablets with cisapride is contraindicated. (See *BOX WARNING*, *WARNINGS*, and *PRECAUTIONS* sections.)
		Concomitant administration of NIZORAL® Tablets with oral triazolam is contraindicated. (See *PRECAUTIONS* section.)

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interactions by health care practitioners. Newer labels frequently include in vitro parameters evaluating the drug's effect on specific cytochrome P450 metabolism and the clinical consequences of the changes in these enzyme activities have on co-administered drugs. In addition, the labels also include the influence of concomitantly administered drugs on the drug itself. Table 2 lists some examples of the labeling language based on in vitro information. Less frequently included in the labels today are transporter information and metabolic interactions based on other noncytochrome P450 enzymes. As the science progresses and technologies in the evaluation become standard, future labeling should include these other types of information.

SUMMARY

As many of the new drugs are to be indicated for patients who receive other drugs or biologies, it is necessary to know the drug interaction potential early on in the development. For compounds eliminated by a single pathway, there is a high probability of drug interaction. The appropriate use of in vitro metabolism (including isozyme characterization) and drug interaction information can provide the basis for the design of confirmatory in vivo studies or obviate the need for further in vivo studies. Further improvement in the in vitro methodologies evaluating other, noncytochrome P450-based metabolilsm/drug interactions and transporterbased interactions should improve our abilities to assess drug-drug interactions for risk/benefit evaluation during drug development and regulatory review.

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6

Drug Transporters

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OVERVIEW

Drug transporters have been a rapidly emerging area in biomedical research for the last 10 years. These drug transporters are proteins located in the intracellular and plasma membranes making up to 2–3% of body total proteins. Drug resistance, low bioavailability, high intersubject variability and gender difference in drug disposition have been linked to drug transporters [1–3]. When a drug is introduced into the body, the transport of a drug from the administered site such as the intestine (absorption) to the target organs such as brain (distribution) and to the organ for metabolism and excretion in the liver and kidney (disposition and elimination) is an important process, in which drug transporters play a critical role. Since this a very broad field, this chapter will discuss the transporters important in ADME (absorption, distribution, metabolism and excretion) of drugs.

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Terminology

Diffusion is a process utilized by lipophilic drugs that can readily permeate the cell membrane down a concentration gradient. Diffusion of polar substances (e.g. nutrients, ions) across the lipid bilayer membrane of cell is limited because the cell membrane acts as a diffusion barrier to the movement of substances into and out of the cell. Cells need to be supplied with polar or charged nutrients (e.g. amino acids, glucose) or to efflux polar molecules for physiological function (e.g. bile acids excretion into the gut). Uptake is a process where the solute is translocated by receptor-mediated or non-receptor-mediated endocytic process (e.g. LDL and transfertin receptors). Transport is a process where the solute is translocated via a membrane protein, which requires a conformational change during the process of translocation. The solute binding site is accessible to only one side of the membrane at any one time. It can be either facilitated (passive) or active. The direction of transport can be influx into or efflux from cells. Channels are tiny pores, which allow ions such as sodium, potassium, chloride, calcium to pass through the membrane. There can be several subtypes of an ion channel for a specific ion. For example, there are several subtypes of potassium channels in cardiac muscle cells. They may undergo conformational change to open or close to traffic and may have specific binding sites for selected solutes. They have binding sites accessible from either side of the membrane. Transport through channels is always facilitated (equilibrative) and much faster than that mediated by transporters.

Classification

Classification of drug transporters is mainly based on energy requirement. **Facilitative transporters** move solutes of a single class (uniporters) down a concentration gradient or an electrical gradient (charged molecules only), which are not energy-dependent, but protein-mediated (e.g., Na^+-independent equilibrative nucleoside transporters). These transporters are saturable, and mediate the influx and efflux of drugs, depending on the direction of the concentration gradient. **Active transporters** can move solutes against a concentration gradient, which is energy-dependent and protein-mediated. There are three types of active transporters: primary, secondary, and tertiary transporters. **Primary transporters** generate energy themselves (e.g., ATP binding cassette or ABC of P-glycoproteins). **Secondary transporters** utilize energy (voltage and ion gradients) generated by a primary active transporter (e.g., Na^+/K^+-ATPase). **Secondary transporters** include symporters and antiporters. **Symporters** translocate two or more different solutes in the same direction (e.g., Na^+-nucleoside

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transporters). **Antiporters** couple the transport of solutes in opposite direction (e.g., H^+/organic cation exchanger in the kidney). **Tertiary transporters** utilize energy indirectly generated by a secondary transporter. An example is the transport of organic anions into kidney epithelial cells in exchange for dicarboxylate ions.

Based on ATP dependence, drug transporters can be divided into two major classes: ATP-binding cassette (ABC) transporters and non-ATP-mediated transporters.

MAJOR TRANSPORTERS

Since there are many transporters in biological membranes, we will only discuss those that are important in pharmacokinetics and pharmacodynamics of drugs.

ATP-Binding Cassette (ABC) Transporters

The nomenclature of ABC transporters was first introduced in 1992 and refers to superfamily of transmembrane proteins [4]. These membrane transporters use ATP hydrolysis as energy to transport a large variety of substrates across cell plasma membranes. ABC transporters are classified based on the sequence and organization of their ATP-binding domains (*n*ucleotide-*b*inding *f*olds, NBFs) rather than their functions. The NBFs contain characteristic motifs (Walker A and B), separated by approximately 90–120 amino acids, found in all ABC transporters. ABC transporters typically contain two NBFs and two transmembrane domains (TMD). The TMDs contain 6–12 membrane-spanning α-helices. The prototypical structure as found in P-glycoprotein (P-gp) consists of 12 membranespanning α-helices and two NBFs. Both ATP binding sites (NBFs) are essential for proper functioning of P-gp [5].

ABC transporter superfamily is divided into seven subfamilies: ABCA/ABC1, ABCB/MDR/TAP, ABCC/MRP, ABCD/ALD, ABCE/OABP, ABCF/GCN20, and ABCG/White. The members of ABC transporters are still growing. Thus far, a total of 51 members have been identified [6]. The major ABC transporters are summarized in Table 1. ABC transporters are located in normal tissues as well as in cancer cell membranes. The genes from three subfamilies are highly expressed in most tumor cells and are attributed to drug resistance, including *ABCB1/ MDR1,* ABCC subfamily genes *(MRP1, MRP2, MRP4, MRP5, MRP6, MRP7),* and *ABCG2/BCRP* gene. Particularly, three ABC transporter proteins, MDR1, MRP1, and BCRP, are found overexpressed in almost all cancer cells responsible for resistance to a large amount of anticancer drugs [7].

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TABLE 1 Representatives of main ATP-Binding Cassette (ABC) transporters

Name	Alias	Human gene nomenclature	Rodent	Location		Substrates	Inhibitors	Transport direction
				Tissue	Subcellular			
MDR1	Pgp PGY1	ABCB1	Mdr1b	Intestines Liver Blood Brain Barriers Placenta	Apical membrane	Daunorubicin Digoxin Fexofenadine Indinavir	Cyclosporine Ketoconazole Quinidine	Efflux
MDR3	PGY3 MDR2 ABC21	ABCB4	Mdr1a	Liver	Apical membrane	Phospholipids		Efflux
BSEP	sPGP PGY4	ABCB11	Bsep	Liver	Apical membrane	Paclitaxel Bile salts		Efflux
MRP1	MRP	ABCC1	Mrp1	Ubiquitous	Basolateral membrane Encosomes	Anionic drug conjugates Methotrexate Estradiol-17β-D-glucuronide	Cyclosporine A Indomethacin Probenecid Sulfinpyrazone	Efflux
MRP2	cMOAT	ABCC2	Mrp2	Liver Intestines Kidney	Apical membrane	Anionic drug conjugates Methotrexate Bilirubin-Glucuronides Acidic bile salts	Furosemide Indomethacin Benzbromarone	Efflux

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MRP3		ABCC3	Mrp3	Liver Intestines Bile ducts Adrenal cortex	Basolateral membrane	Anionic drug conjugates Methotrexate Estradiol-17β-D-glucuronide	—	Efflux
MRP4		ABCC4	Mrp4	Many tissues	Apical membrane	Organic anions Estradiol-17β-D-glucuronide Cyclic nucleotide analogs	Probenecid Dipyridamole	Efflux
MRP5		ABCC5	Mrp5	Ubiquitous	Basolateral membrane	Organic anions Cyclic nucleotide analogs	Probenecid Sulfinpyrazone Zaprinast Trequisin Sildenafil	Efflux
MRP6		ABCC6	Mrp6		Basolateral membrane	BQ-123 (peptide)	—	Efflux
BCRP1	MXR1 ABCP	ABCG2	mBerp1	Breast Placenta Liver Endothelium	Apical membrane	Amphipathic drugs	—	Efflux

Note. MDR: multidrug resistance; Pgp: P-glycoprotein; MRP: multidrug resistance-associated protein; BCRP: Breast cancer resistance proteins.

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P-glycoproteins (P-gp)

Two genes in ABCB subfamily, *MDR1 (ABCB1)* and *MDR3 (also called MDR2, AECB4)* encode P-glycoproteins (P-gp) [8, 9]. Both the protein products are efflux transporters. However, *MDR3* translocates endogenous phosphatidylcholine as the main function [10, 11]. Generally, P-gp only refers to *MDR1* gene products. P-gp contains 1280 amino acids, which are translated from 28 exons of their genes [12]. *MDR1* and *MDR3* are 76% identical in gene sequence [13]. Two mouse genes *Mdrla* and *Mdr1b* correspond to the human *MDR1* gene. The human *MDR1* and these mouse *Mdr* genes share 88% identity in gene sequence and have similar function. *MDR1* gene was the first cloned in ABC transporter family [14]. P-gp (*MDR1* gene product) is the best-characterized ABC drug efflux pump. P-gp plays an important role in multidrug resistance to anticancer drugs in cancer cells and in the transport of hydrophobic substrates including endogenous compounds such as lipids, steroids, and a wide variety of drugs. P-gp has been recognized as one of the important systems to affect bioavailability and disposition of drugs. More details of the function of P-gp will be described later. *MDR3* is mainly expressed in the bile canalicular membrane of the hepatocytes to transport endogenous phospholipids from the hepatocyte to the bile. Recently *MDR3* was found to transport some hydrophobic drugs as well [15].

Multidrug Resistance Associated Proteins (MRPs)

These transporters belong to ABCC subfamily and play a significant role in drug resistance in cancer cells [16]. *MRP1* is expressed in tumor cells and confers resistance to anticancer drugs, such as doxorubicin, daunorubicin, vincristine, and colchicines [17]. *MRP2* is expressed in canalicular cells in the liver [18]. It functions as the major efflux pump of organic anions from the hepatocyte into the bile. Dubin-Johnson syndrome is attributed to a mutation of *MRP2* gene [19]. MRP3 protein is expressed primarily in the liver. Similar to *MRP2, MRP3* confers the ability to efflux organic ions [20]. *MRP4* gene is expressed at low levels in many tissues [21]. Overexpression and amplification of the *MRP4* gene is found in cancer cell lines resistant to nucleoside analogues such as azidothymidine monophosphate. Thus, *MRP4* may be an important factor in the resistance to nucleoside analogues [22]. Because these drugs are important antiviral and anticancer agents, this has importance in therapies for HIV1 infection and cancer chemotherapy. *MRP5* gene is ubiquitously expressed in many tissues. It is closely related to the *MRP4* gene and confers resistance to nucleoside analogues [23]. *MRP6* gene is principally expressed in the liver and kidney [24]. Human MRP6 protein is present in isolated membranes and can transport glutathione conjugates including LTC4 [25]. Genetic polymorphism in *MRP6* gene has

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been linked with abolished transport activity and disease status such as abnormal lipid levels [26].

Breast Cancer Resistance Protein (BCRP)

BCRP encoded by *ABCG2* gene is a half transporter expressed in normal tissue [5]. BCRP functions as an efflux transporter serving as a cellular defense mechanism. Indeed, BCRP and P-gp appear to have considerable overlap in substrate selectivity. BCRP is highly expressed in the trophoblast cells of the placenta, which may suggest a potential role in the blood-placenta barrier [27]. BCRP is also expressed in many resistant cancer cell lines, which may play a major role in multi-drug resistance in response to mitoxantrone and anthracycline exposures [28, 29]. Inhibition of these ABC drug transporters represents a potential strategy for preventing the development of drug-resistance and increasing anticancer drug accumulation in tumors.

Non-ATP-Mediated Transporters

Several non-ATP-mediated membrane transporter families have been identified, which include organic anion transporting polypeptides (rodent: oatp, human: OATP), organic anion transporters (rodent: oat, human: OAT), organic cation transporters (OCT), and peptide transporters (rodent: pept, human: PEPT). These transporter families play important roles in the disposition and elimination of a variety of endogenous substances, drugs, and their metabolites from the body. The representative members of these families are summarized in Table 2.

Organic Anion Transport Polypeptide (OATP)

Currently, at least nine human OATPs have been identified [30, 31]. OATPs are a group of membrane solute carriers with a wide spectrum of amphipathic substrates [32]. Although some important members of this transporter family are selectively expressed in human livers, most human OATPs are expressed in multiple tissues including the blood-brain barrier (BBB), choroid plexus, heart, intestine, kidney, and placenta [33–38]. Only some of the OATPs so far identified have been characterized in detail at the functional, structural, and genomic levels. Many members of this transporter family represent polyspecific organic anion carriers for transport of a wide range of amphipathic organic solutes. Depending on which side of membrane they are located, OATPs may be responsible for influx or efflux of a wide variety of amphipathic endogenous substances, drugs, and their metabolites.

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TABLE 2 Representatives of the Major Human Non-ABC Transporters

Name	Alias	Human gene symbol	Localization		Substrates	Inhibitors	Transport direction
			Tissue	Subcellular			
OATP-A	OATP, OATP1	SLC21A3	Liver Brain	Basolateral membrane	Fexofenadine Ouabain N-methyl-Quinidine Rocuronium	Rifamycin	Uptake
OATP-B	OATP-RP3	SLC21A9	Liver Kidney Intestine Pracenta	Basolateral membrane	Estrone-3-sulfate Benzylpenicillin	Rifamycin	Uptake
OATP-C	LST-1 OATP2 OATP6	SLC21A6	Liver	Basolateral membrane	Benzylpenicillin Digoxin Methotrexate Pravastatin Rifampin	Rifamycin Rifampin	Uptake
OATP-D	OATP-RP3	SLC21A11	Ubiquitous	Basolateral membrane	Benzylpenicillin Estrone-3-sulfate	—	Uptake
OATP-E	OATP-RP1	SLC21A12	Ubiquitous	Basolateral membrane	Estrone-3-sulfate Estradiol-17β-D-glucuronide	—	Uptake
OATP-F	OATP-RP5	SLC21A14	Brain Testis	Basolateral membrane	Estrone-3-sulfate Estradiol-17β-D-glucuronide	—	Uptake
OATP8	LST-2	SLC21A8	Liver	Basolateral membrane	Digoxin Methotrexate Ouabain Rifampin Estrone-3-sulfate Estradiol-17β-D-glucuronide	Rifamycin Rifampin	Uptake

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OAT1	SLC22A6	Renal proximal tubules	Basolateral membrane	Cidofovir	Probenecid Furosemide Indomethacin Cilastatin	Uptake
OAT2	SLC22A7	Renal Henle's loop	Apical membrane	Methotrexate	Ketoprofen Bumetanide Enalapril Cefoperazone	Uptake
OAT3	SLC22A8	Renal proximal tubules	Basolateral membrane	Methotrexate	Betamipron Cilastatin Ibuprofen Diclofenac Indomethacin Corticosterone Quinidine Probenecid	Uptake
OAT4	SLC22a8	Renal proximal tubules	Apical membrane	Estrone-3-sulfate	Bumetanide Ibuprofen Diclofenac Indomethacin Corticosterone Furosemide Probenecid	Efflux
OCT1		Renal proximal tubules	Basolateral membrane			Uptake
OCT2		Renal proximal tubules	Basolateral membrane			Uptake
OCT3		Placenta	Fetal facing basal membrane	Neurotoxin Guanidine	Cimetidine Serotonin Desipramine	Uptake

Note: OATP: organic anion-transporting polypeptide; OAT: organic anion transporter; OCT: organic cation transporter.

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Organic Anion Transporter (OAT)

Human OATs play important roles especially in the elimination of a variety of endogenous substances, drugs, and their metabolites from the liver and kidney. So far, five OAT members have been identified [39–43]. Structurally, OATs are membrane proteins with 12 putative membrane-spanning domains and function as sodium-independent exchangers or facilitators [44]. OATs are multispecific organic anion transporters, the substrates of which include both endogenous (e.g., cyclic nucleotides, prostaglandins, urate, dicarboxylates) and a wide variety of clinically important anionic drugs, such as ß-lactam antibiotics, diuretics, NSAIDs, anti-HIV therapeutics, anti-tumor drugs, and angiotensin-converting enzyme inhibitors [45–48]. The most commonly used model substrate for OAT studies is paraaminohippuric acid (PAH). Therefore, the OAT system has alternatively been called the PAH transport system. All members of the OAT family are expressed in the kidney, while only some are expressed in the liver, brain, and placenta [49–51]. The OAT family represents the renal secretory pathway for organic anions and is also involved in the distribution of organic anions in the body [52]. OAT-K1, together with MRP2 and OATP1, may contribute to the efflux of organic anions into luminal side of renal proximal tubules. OAT-K1 is a Na^+-dependent transporter system, whereas OAT2, OAT3, and OAT4 are Na^+-independent transporters, whose function is to uptake organic anions into cells [53]. OATs may play a role in drug interactions as well. It has been reported that concurrent use of methotrexate with acidic drugs, such as NSAIDs, ß-lactam antibiotics, causes severe suppression of bone marrow, which seems to be related to the competitive inhibition of the renal OAT system [54].

Organic Cation Transporters (OCT)

Three members of OCT have been reported. OCT1, OCT2, and OCT3 transporters are electrogenic, Na^+-independent, and pH-independent facilitated diffusion systems responsible for the uptake of organic cations into the cells [55]. In small intestine, liver, and segments of rat kidney proximal tubules, OCT1 is localized in the basolateral membranes of polarized epithelial cells [56]. The expression of OCT2 is more tissuespecific. Human OCT2 is detected mainly in the kidney with some expressed in brain and small intestines [57–59]. Human OCT2 in brain may help to reduce the background concentration of basic neurotransmitters and their metabolites [60].

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TISSUE AND CELLULAR LOCALIZATION

The tissue distribution of transporters has been studied using different techniques. Consistent with their potential role in detoxification processes and physiological functions, transporters are expressed in various tissues as demonstrated in human normal tissues as well as in human cancer cell lines. Certain transporters show a more restricted tissue expression pattern (*MDR3, BSEP, OATP-A, OATP-C,* and *OATP8*) while others can be detected in almost every tissue that has been investigated (e.g., *MDR1, OATP-B, OATP-D, and OATP-E*). This indicates that some transporters have organ-specific functions while others might be involved in more housekeeping functions.

Intestines

P-gp is expressed in the luminal membrane of intestinal mucosal epithelium. Several efflux pumps such as BCRP, MRP2, and MRP4 are also highly expressed in the intestinal mucosal epithelial cells. However, some of MRPs are expressed at basolateral membrane of intestinal epithelium, such as MRP1, MRP3, and MRP5 (Fig. 1). The abundance of P-gp expression varies in different intestinal sections. The expression of P-gp increases with distance. (The lowest amount of P-gp is located in stomach, highest in colon, and medium in jejunum/ileum [61], exactly opposite to the expression of CYP3A4/5.) CYP3A4/5 expression decreases longitudinally [62].

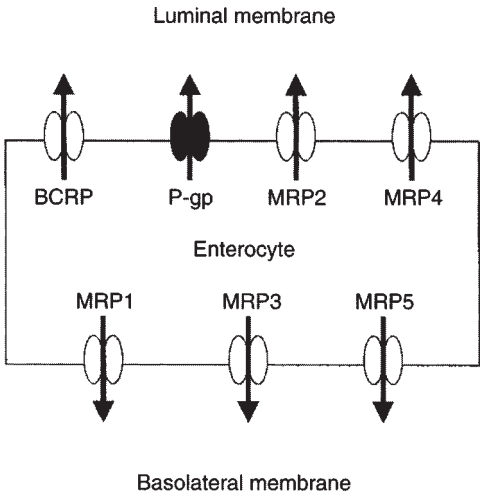

FIGURE 1 Schematic representation of selected ABC transporters in the intestinal membrane.

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Liver

Liver is an important organ for metabolism of numerous endogenous and exogenous compounds, a process in which many transporters are involved. Hepatic uptake of organic anions, cations, and bile salts is supported by transporters in the basolateral (sinusoidal) membranes of hepatocytes including OATPs, OATs, and OCTs. ATP-binding cassette transporter proteins in the canalicular membranes of hepatocytes mediate the hepatic efflux of drugs, bile salts, and metabolites against a steep concentration gradient from liver to bile, which includes the MDR1 and MDR3, MRP2, and BSEP. However, MDR3 is mainly responsible for the transport of endogenous phospholipids though a recent report indicated that MDR3 may transport some drugs [63]. These transporters play essential roles in transporting, metabolizing, and excretion of bile salts, xenobiotics, and environmental toxins (Fig. 2).

Kidney

Multiple organic anion transporters play important roles in the elimination of a variety of endogenous and exogenous compounds, and their metabolites from the body. Several families of multispecific organic anion transporters mediating the renal elimination of organic anions have been identified. Members of the organic anion transporter (OAT), organic anion transporting polypeptide (OATP), multidrug resistance protein (MRP),

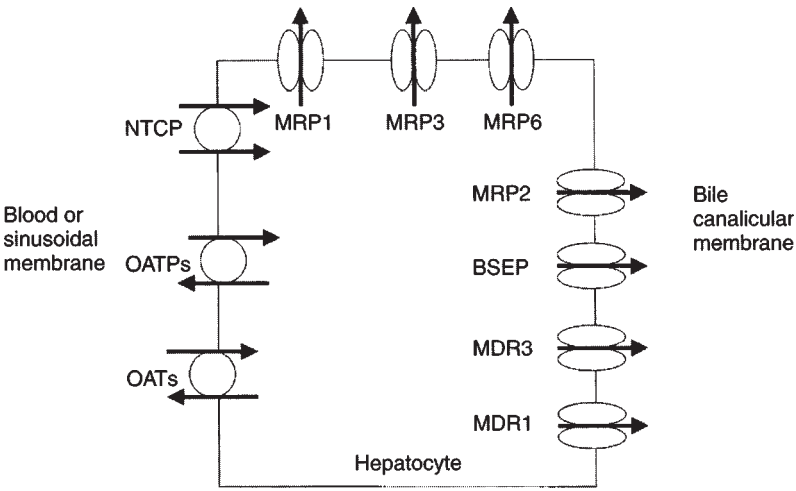

FIGURE 2 Schematic representation of selected drug transporters in hepatocytes.

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sodium–phosphate transporter (NPT), and peptide transporter (PEPT) families have been identified in the renal proximal tubules. Uptake of organic anions (OA^-) across the basolateral membranes of renal epithelial cells followed by efflux into urine across the apical membrane is mediated by the Na^+-dependent organic transporter, OAT1 and the Na^+-independent organic transporter, perhaps OAT3. The function of MRP6 at the basolateral membrane is unknown. Efflux across the apical membrane of organic anions is through low-affinity anion exchange and/or facilitated diffusion, and a Na^+-independent ATP-driven system. The luminal membrane contains various efflux transporter proteins including OATK1/K2, OAT4, NPT, MRP2, and MRP4. The luminal membrane also contains various uptake transporters such as OATP1, PEPT 1/2 (Fig. 3).

Brain

The brain is protected against drugs and toxins by the two drug-permeability barriers: the BBB and the blood–cerebrospinal fluid (CSF) barrier (BCSFB). The BBB is primarily formed by the endothelium of the blood capillaries in the brain. P-gp is expressed in the luminal plasma membrane of capillary endothelial cells and plays a significant role in restricting the brain permeability of drugs [64].

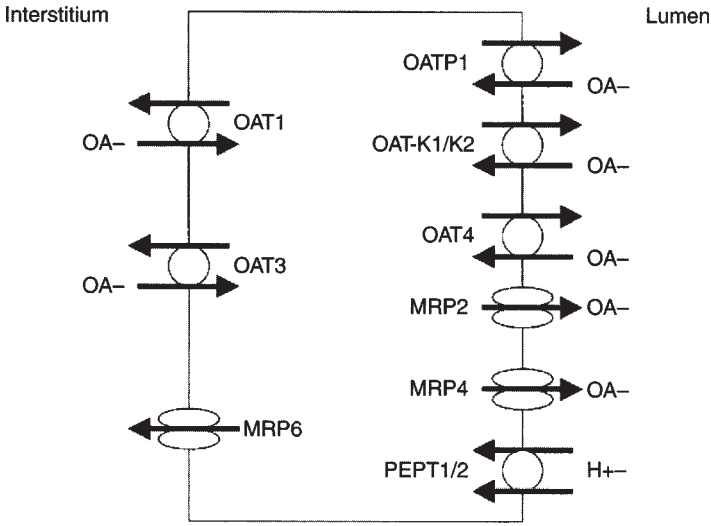

FIGURE 3 Schematic representation of selected renal drug transporters.

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P-gp is expressed to a great extent in the apical (luminal) plasma membranes of these capillary endothelial cells, conferring an apical-to-basal transepithelial permeation barrier to drugs. MRP1 localizes basolaterally, conferring an opposing basal-to-apical drug-permeation barrier. Together, these transporter proteins may coordinate secretion and reabsorption of endogenous substrates and therapeutic drugs into and out of the central nervous system [65].

Recently, some other transporter proteins including MRPs, OATP, and OAT have been also reported to exist in the BBB and the BCFSB [66, 67].

Placenta

P-gp is expressed at the brush border membrane of the syncytiotrophoblast. The expression appears to be higher early in gestation compared with term placenta [68, 69]. Absence or pharmacological inhibition of placental P-gp profoundly increases fetal drug exposure. Intravenous administration of radioactive digoxin, saquinavir, and paclitaxel to pregnant dams resulted in 2.4-, 7-, or 16-fold more drug in fetuses with *mdrla* (-/-)(-/-) *1b* (-/-)(-/-) than the wild-type fetuses. Placental P-gp could be completely inhibited by PSC833 or GG918 when given to heterozygous dams indicating that the placental drug-transporting P-gp is of great importance in limiting the fetal penetration of various potentially harmful or therapeutic compounds, and demonstrate that this P-gp function can be abolished by pharmacological means [70].

The mRNA levels of various transporters in rat placenta were assessed during late-stage pregnancy. Sixteen mRNAs of various transporters were expressed in placenta at concentrations similar to or higher than that in maternal liver and kidney. They include *Mdrla* and *1b, Mrpl, Mrp5, Oct3* and *Octn1, Oatp3,* and *oatp 12* [71]. The abundance of these mRNA transcripts in placenta suggests a role for these transporters in placental transport of endogenous and exogenous compounds. In human placenta, OATP-B has been detected in the trophoblast at the basal membranes where it may play a role in transporting natural substrates (e.g., steroid hormone conjugates) from the fetal circulation into the trophoblast [72].

FUNCTION OF P-GLYCOPROTEINS

P-glycoprotein is the product of multidrug resistance gene family, *MDR1* and *MDR3*. P-gp encoded by *MDR3* is expressed at the canalicular membrane of hepatocytes and is responsible for transporting phospholipids into bile ductules although a recent report has indicated that it may also transport some drugs. P-gp, *MDR1* product, is expressed in many normal

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tissues including intestines, liver, brain, placenta, and testis though it was first discovered from cancer cells as a multidrug resistance protein. P-gp acts as an efflux pump by translocating substrates from the intracellular to the extracellular compartment.

Substrates, Inhibitors, and Inducers

P-gp has an ability to transport drugs diverse in chemical structure from different therapeutic classes (Table 1). Another striking feature is an overlap in substrates between P-gp and CYP3A4/5. These two substrate-sharing systems may serve as protective physiological barriers to limit harmful exposure to exogenous compounds.

Pharmacokinetic Implication

The high expression of P-gp in many tissues has made P-gp an additional physiological barrier to protect the body from the exposure to toxins and xenobiotics. Numerous studies have shown that P-gp plays an important role in the fate of absorption, distribution, metabolism, and excretion of drugs.

P-gp was first detected in certain cancer cells associated with the phenomenon of multiple drug resistance (MDR). However, it is now known that P-gp is highly expressed in normal tissues. In fact, P-gp is located in the apical domain of the enterocyte of the lower gastro-intestinal tract (jejunum, duodenum, ileum, and colon), thereby limiting the absorption of drug substrates from the gastro-intestinal tract. In other organs such as the liver and kidney, expression of this transporter at the apical membrane of hepatocytes and proximal tubular cells in kidney results in enhanced excretion of drug substrates into bile and urine respectively. P-gp is an important component in the BBB, limiting the CNS entry of a variety of drug substrates. P-gp is also found in other tissues known to have tissue–blood barriers, such as placenta and testis.

Absorption

Drug absorption is a collective result from passive diffusion across intestinal membranes down a concentration gradient, intestinal metabolism, and P-gp efflux from the epithelial cells into the intestinal lumen. The effect of P-gp on drug absorption has been demonstrated using *Mdr* knockout mice and studies with P-gp inhibitors. Many clinically significant drug interactions are due to the inhibition of P-gp in the intestines.

After intravenous and oral administration of paclitaxel, the AUC was twofold and sixfold higher in *Mdrla* (-/-) mice compared to the wild-type

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(wt) mice. Oral bioavailability of paclitaxel in *Mdrla* (-/-) and wt mice was 35% and 11% respectively. Biliary excretion of the drug was not different between the two groups of mice. After oral administration, 87 and 2% of the dose were found in the feces as paclitaxel in wt and *mdrl* a (-/-) mice suggesting substantial change in the extent of absorption of the drug when the effect of P-gp is removed [73].

Oral absorption of paclitaxel was increased when wt mice were cotreated with P-gp inhibitors, cyclosporine, or SDZ PSC 833. The oral AUC of paclitaxel was dramatically increased from 735 to 8066ng.h/ml when PSC833 was administered [74]. Concurrent drug therapy of P-gp inducers may decrease drug absorption. After two weeks of treatment with rifampin, the AUC of a single oral dose of digoxin was significantly reduced, due to the induction of intestinal P-gp [75].

Distribution

As indicated earlier, the blood, brain, and the placental barriers are obstacles for a drug to reach the privileged compartments of the brain and the fetus.

After intravenous administration of digoxin and cyclosporine to *Mdrla* (-/-)(-/-) and wt mice, the ratio, (-/-):(+/+), of brain concentrations of digoxin and cyclosporine in these mice was about 35 and 17, while the plasma concentration ratio was only 1.9 and 1.4 respectively. Thus, mice without P-gp have increased concentrations of digoxin and cyclosporine in the brain [76].

Modulation of P-gp may result in an increase in the CSF levels of the protease inhibitors and this may have clinical implications. The disposition of protease inhibitors, indinavir, nelfinavir, and saquinavir was studied in *Mdrla* (-/-) and wt mice. Labeled compounds were administered intravenously and orally. After IV administration, there was no significant difference in plasma concentrations of total radioactivity at 4h, but the brain concentrations were considerably elevated in the *Mdrla* (-/-) mice. The brain concentration to plasma concentration ratio was the highest for nelfinavir and lowest for indinavir and saquinavir. After oral administration, radioactivity in the plasma was higher at 4 h in *Mdrla* (-/-) mice for all the three drugs [77]. The efflux of protease inhibitors from the brain in wt mice can be inhibited by the P-gp inhibitor, LY335959 [78]. OC144–093, a novel, extremely potent inhibitor of P-gp, does not inhibit multidrug resistance-associated protein (MRP1). This compound is not metabolized by cytochrome P4503A4, 2C. The enhancement of BBB penetration of antiepileptic drugs (AEDs) can be achieved with co-administration of OC144–093 [79]. The presence of P-gp in the placenta limits fetal exposure to several compounds, but inhibition of P-gp can

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enhance the fetus concentrations of protease inhibitors and consequently may aid in the protection of the fetus from HIV infection.

Metabolism

Cytochrome P450s are expressed in the luminal membranes of intestines. These CYP enzymes are mainly CYP3A4/5 [62, 80–83]. The co-expression of P-gp and CYP3A4/5 and the interplay between P-gp and CYP3A4/5 in enterocytes result in longer residence time in enterocytes for drugs, potentially resulting in reduced bioavailability of certain drugs [84]. Since P-gp and CYP3A4/5 share common inducers, such as rifampicin and St. John's wort [85], increased expression of both systems may result in reduced bioavailability of certain therapeutic agents.

Excretion

As described previously, P-gp is highly expressed in the hepatic bile canalicular membrane and renal proximal tubule luminal membrane. Inhibition of P-gp may result in changes in biliary excretion or renal proximal tubule excretion or both, depending on pharmacokinetic characteristics of the individual drug.

Digoxin is mainly eliminated by the kidney (~60%) and the rest by biliary secretion. Its renal clearance is greater than the filtration clearance indicating secretion of the drug by the kidney tubules. Kidney epithelial cell lines expressing human *MDR1* transport digoxin from basal to the apical membrane, and this transport is inhibited by cyclosporine [86]. In another cell line expressing *MDR1,* the potency of inhibition by the azoles decreased from itraconazole > ketoconazole >fluconazole [87]. A concomitant use of itraconazole increases the serum concentrations of digoxin. In a study with ten healthy volunteers, either 200 mg itraconazole or placebo was given orally once a day for five days. On day 3, each volunteer ingested a single 0.5-mg oral dose of digoxin. Digoxin AUC (0–72) was approximately 50% higher during the itraconazole phase than during the placebo phase. The renal clearance of digoxin was decreased by about 20% ($P<0.01$) by itraconazole. The decreased renal clearance of digoxin during the itraconazole phase may explain increased concentrations of digoxin during their concomitant use due to the inhibition of P-gp-mediated digoxin secretion in the renal tubular cells [88].

The effects of quinine and quinidine on the biliary and renal clearances of digoxin were investigated in healthy subjects. Digoxin was given alone and with concomitant administration of quinine or quinidine. Quinine and quinidine markedly reduced the steady-state biliary clearance of digoxin by about 35 and 42% respectively, while the steady-state renal clearance of digoxin was reduced significantly only by quinidine (29%) [89]. In a study

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of the effect of verapamil on the steady-state digoxin plasma concentrations, biliary and renal clearance of digoxin, the steady state concentration of digoxin was increased by 44%, and biliary clearance of digoxin was decreased by 43%, but renal clearance was unaffected, which may indicate that similar to quinine, verapamil only inhibits the transporters of biliary system [90].

Genetic Polymorphism

Although the genetic polymorphism of human *MDR1* gene has been reported since late 1980s [91, 92], the impact of *MDR1* genetic polymorphism on drug pharmacokinetics was highly contraversial. Hoffmeyer et al. conducted a systemic screening for *MDR1* polymorphism and detected 15 single nucleotide polymorphisms (SNPs). An SNP in exon 26 of the *MDR1* gene, *C3435T* (a silent mutation with no amino acid change), was correlated with P-gp protein levels and digoxin plasma concentrations after oral administration of the drug. Individuals homozygous for the T allele have four fold lower P-gp expression and higher digoxin plasma concentrations compared with CC individuals [93]. However, a later report showed the subjects with genotype TT had lower digoxin plasma concentrations in a much larger subject pool, a result opposite to the previous report [94]. Additional reports showed that there is no correlation between the genotype *C3435T* and pharmacokinetic profiles of P-gp substrates [95, 96]. There may not be a solid correlation between genotype *C3435T* and its phenotype because this may be linked with other functional polymorphism in the gene.

Additional functional variants of *MDR1* have been disclosed. The functional relevance of nonsynonymous SNP (*G2677T, Ala893Ser*) in exon 21 was reported. In vitro expression of *MDR1* encoding *Ala893* or a sitedirected *Ser893* mutation indicated the enhanced efflux of digoxin by cells expressing the *MDR1-Ser893* variant. In vivo functional relevance of this SNP was assessed with the P-gp drug substrate fexofenadine. Subjects with homozygous *Ala893* showed higher fexofenadine plasma exposure than those with homozygous *Ser893* [97].

So far, at least 30 SNPs have been reported in the *MDR1* gene. Human in vivo studies on *MDR1* genotype-related pharmacokinetics have been reported. However, results were not always consistent. More work needs to be done to establish the correlation between the genotype and the phenotype. Haplotypes of these SNPs may allow a definition of this correlation.

Significance in Drug Development

Because P-gp functions as an efflux pump in cancer cell membranes which contributes resistance to many anticancer drugs leading to failure of

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chemotherapy. Although a few potent P-gp inhibitors are being developed, the efficacy has not been very satisfactory [98–100]. A challenge facing pharmaceutical scientists is to develop tumor-specific P-gp inhibitors to reverse the function of P-gp and to reach adequate accumulation of anticancer drugs in cancer cells [101]. The same challenge exists for targeted drug delivery where P-gp expression is abundant. One of the examples is the delivery of anti-epileptic drugs to the central nervous system [102]. P-gp in the BBB is the main obstacle to deliver drugs into the central nervous system. To develop a tissue-targeted P-gp inhibitor or delivery system would provide an additional strategy to treat many CNS diseases without increased exposure to peripheral tissues.

The determination of drug candidates as substrates, inhibitors, or inducers of cytochrome P450s has been a necessary step to meet the regulatory authorities' requirements. Lately, whether or not the drug candidate is a substrate, an inhibitor, or an inducer of P-gp has received a great attention to because of potential drug interaction issues. Many drugs are substrates of cytochrome P450 3A and P-gp, and their disposition is markedly affected by concurrent treatment with inducing agents, such as rifampin and St. John's wort. The inducing effects of both these agents have been reported to substantially decrease plasma concentrations and efficacy of substrate drugs including cyclosporine [103,104], protease inhibitors [105,106], oral contraceptives [107], and digoxin [108]. These drugs are substrates of cytochrome P4503A4 and/or substrates of P-gp. Both rifampin and St. John's wort are potent inducers of both *CYPs* and *MDR1* through a common mechanism that is bound to the pregnane X receptor (PXR) [85]. The screening of PXR ligands has become a useful tool in drug development to select molecules with a lesser capacity to induce drug-metabolizing enzymes and *MDR1* [109].

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7

Principles, Issues, and Applications of Interspecies Scaling

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INTRODUCTION

This chapter describes different techniques and approaches to predict pharmacokinetic parameters from animals to humans during drug development. These techniques are useful and if used with proper understanding, it will be time and cost effective. The chapter illustrates the advantages and the limitations of allometric scaling.

Allometry is based on the assumption that the relationship between anatomy and physiologic functions is similar among mammalian species [1, 2]. Over the years, allometry has become a useful tool for correlating pharmacokinetic parameters with body weight from different animal species. By establishing such a correlation, one can predict pharmacokinetic parameters in humans which can be useful during drug development. Interspecies scaling to predict pharmacokinetic parameters in humans can be performed by two approaches:

i. physiologically based method (PB-PK),
ii. empirical allometric method.

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Physiological method, however, has found only limited use in drug discovery and development, because this approach is costly, mathematically complex, and time consuming.

On the other hand, the allometric approach though empirical, is less complicated and easy to use than the physiologically based method. The anatomical, physiological, and biochemical similarities among animals can be generalized and expressed mathematically by the allometric equation. The allometric approach has been based on the power function, as the body weight from several species is plotted against the pharmacokinetic parameter of interest on a log-log scale. The power function is written as follows:

$$Y=aW^b \tag{1}$$

where Y is the parameter of interest, W is the body weight, and a and b are the coefficient and exponent of the allometric equation, respectively. The log transformation of Eq. (1) is represented as follows:

$$\log Y=\log a+b \log W \tag{2}$$

where log a is the y-intercept, and b is the slope.

Besides, using the power function to establish a relationship between a pharmacokinetic parameter of interest and body weight, the power equation has also been used to establish relationship between body weight and physiologic parameters such as liver weight, liver blood flow, kidney weight, kidney blood flow, and glomerular filtration rate of several species including humans [3].

Using allometric approach, many pharmacokinetic parameters such as clearance (CL), volume of distribution (V), elimination half-life ($t_{1/2}$), and absolute bioavailability (F) from animals to humans have been predicted [3]. The following sections will describe several allometric approaches to predict these parameters from animals to humans.

Clearance

Clearance is the most important pharmacokinetic parameter. The knowledge of clearance is especially very important during drug discovery or screening process, since drugs which are eliminated quickly may have a low absolute bioavailability and may not be suitable for further investigation. Clearance can also play an important role for the selection of the first-time dosing in humans [as inverse of clearance indicates the total exposure, area under the curve (AUC) of a drug]. Therefore, considering the importance of clearance, over the years, a lot of attention

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has been focused in order to improve the performance of allometry to predict clearance. In a given species, clearance can be estimated by the following equation:

$$\text{Clearance} = \frac{\text{Dose}}{\text{AUC(0-infinity)}} \tag{3}$$

where AUC is the area under the plasma concentration vs. time curve calculated by trapezoidal rule and then extrapolated to infinity [4].

A survey of the literature [3] indicates that simple allometry [Eq. (1)] alone is not adequate to predict clearance in humans from animal data. Therefore, many approaches have been suggested to improve the prediction of clearance in humans from animals. These approaches can be summarized as follows:

Simple Allometry

This approach is based on Eq. (1) or (2), where the clearance of several species is plotted against body weight.

Maximum Life-span Potential (MLP)

This approach is based upon the concept of neoteny [5] where the clearance is predicted on the basis of species weight and maximum life-span potential (MLP).

$$\text{CL}=a\ (\text{MLP}\times\text{Clearance})^{b}/8.18\times10^{5} \tag{4}$$

where 8.18×10^{5} (in hours) is the MLP value in humans.

MLP in years is calculated from the following equation as described by Sacher [6]:

$$\text{MLP (years)}=185.4\ (\text{BW})^{0.636}\ (\text{W})^{-0.225} \tag{5}$$

where both brain weight and body weight are in kilograms. In Table 1, the MLP values of several species have been presented.

Although Boxenbaum and Dilea [7] mention that neoteny is a trivial biologic phenomena with no real relationship to the phase I oxidative metabolism of drugs, MLP appears to be a useful tool that can be used to predict clearance in humans under specific conditions.

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TABLE 1 Mean Body and Brain Weight and the Estimated MLP in Several Species

Species	Body weight (kg)	Brain weight (kg)	MLP in years	MLP = $\times 10^5$ hrs
Mouse	0.03	0.000418	2.8	0.25
Rat	0.25	0.00174	4.5	0.39
Guinea pig	0.30	0.00362	6.8	0.60
Rabbit	2.75	0.0115	8.7	0.76
Cynomolgus monkey	3.75	0.0424	18.5	1.62
Baboon	5	0.0312	14.3	1.25
Rhesus monkey	6	0.0768	24.2	2.12
Dog	12	0.0754	20.4	1.79
Pig	30	0.05	12.8	1.12
Sheep	58	0.11	18.3	1.60
Human	70	1.53	93.4	8.18
Horse	400	0.57	33.6	2.95

The body weight and brain weight taken from Ref. [8]. The body weight of animals has been slightly modified as per Ref. [8].

Two-term Power Equation

This approach as suggested by Boxenbaum and Fertig [8] uses a two-term power equation based on brain weight and body weight to predict intrinsic clearance of drugs which are primarily eliminated by phase I oxidative metabolism.

$$\text{CL}=A\,(\text{body weight})^{b}\,(\text{brain weight})^{c} \tag{6}$$

where A is the coefficient and b and c are the exponents of the allometric equation. Using Eq. (12), one can also predict unbound intrinsic clearance of drugs.

Product of Brain Weight and Clearance

Mahmood and Balian [9, 10] suggested the use of the product of brain weight and clearance in order to improve the predictive performance of allometric scaling for clearance.

$$\text{CL}=(\text{BW}\times\text{Clearance})^{b}/1.53 \tag{7}$$

where both brain weight (BW) and body weight (W) are in kilograms.

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Mahmood and Balian [9] examined the above mentioned four methods to predict the clearance of seven antiepileptic drugs in humans from data obtained from at least three animal species. From the study, the authors concluded that all the abovementioned methods can predict clearance with different degrees of accuracy. However, the random use of these approaches is of no practical value and it is important to identify the suitability of a given approach. In a separate study, Mahmood and Balian [10] evaluated three methods (except the two-term power equation) to predict the clearance of 40 drugs in humans from data obtained from at least three animal species. In this study, the exponents of clearance ranged from 0.35 to 1.39. From this study the authors concluded that there are specific conditions under which only one of the three methods can be used for reasonably accurate prediction (arbitrarily selected, if the difference between predicted and observed values is 30% or less) of clearance:

i. if the exponent of the simple allometry is within 0.55 to 0.70, simple allometry will predict clearance more accurately than CL×MLP or CL×Brain Weight.
ii. if the exponent of the simple allometry lies between 0.71 and 1.0, the CL×MLP approach will predict clearance better compared to simple allometry or CL×Brain Weight.
iii. if the exponent of the simple allometry is $\geqslant$1.0, the product of CL×Brain Weight is suitable approach to predict clearance in humans compared to the other two methods.

It was also mentioned by Mahmood and Balian that if the exponents of the simple allometry are greater than 1.3, it is possible that the prediction of clearance from animals to man may not be accurate even using the approach of CL×Brain Weight, and if the exponents of simple allometry is below 0.55, the predicted clearance may be substantially lower than the observed clearance. However, this "rule of exponents" is not rigid and there may be some exceptions where this rule may not work. Furthermore, one should also use the scientific judgement when the exponents of simple allometry are on the borderline (e.g., 0.70 vs. 0.71).

The exponents of allometry are of vital importance and three important properties regarding the allometric exponents for clearance should be noted:

1. The exponent of clearance will vary with the species used in the scaling:

For a given drug the exponents of clearance is not universal. The exponents of simple allometry will depend on the species used in the allometric scaling. For example, when clearance of ethosuximide was scaled from mice, rat, and dog [11], the clearance was predicted

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accurately by a simple allometric equation (exponent=0.51, r=0.880). The predicted clearance of ethosuximide was 10 mL/min, whereas the observed clearance was 12mL/min. Using the clearance data from rat, rabbit, and dog [12], the exponent of simple allometry was 1.01 (r-0.953). The predicted clearance using simple allometry, MLP, and the product of brain weight and clearance was 44 mL/min, 15 mL/min, and 10 mL/min, respectively. Scaling of theophylline [10] provided similar observation as that of ethosuximide. When clearance data were scaled from mice, rat, rabbit, and dog, the clearance was predicted accurately by a simple allometric equation (exponent=0.657, r=0.954). Using the clearance data from rat, rabbit, and dog, accurate prediction of clearance was only possible by using MLP (exponent from simple allometric equation was 0.905, r=0.984). This indicates that the exponents of clearance based on allometric principles depend on the species used in the scaling and this phenomena will be true for any given drug. These examples also indicate the importance of the "rule of exponents." It is also obvious that the random use of simple allometry, MLP, or brain weight approach will not help to improve the prediction of clearance from animals to humans.

2. The exponents of simple allometry have no physiological meaning:

The normalization of clearance by MLP or brain weight is a mathematical manipulation which may not be associated with the physiology of the species used in the scaling. As the exponents of the simple allometry get larger the predicted clearance becomes comparatively higher than the observed clearance. The predicted clearance values will be on the order of simple allometry>MLP×CL>brain weight×CL. Furthermore, the application of MLP and the product of brain weight and clearance is not limited to the extensively metabolized drugs rather can also be applied to drugs which are eliminated by renal route.

3. Concept of a fixed exponent of 0.75 for clearance:

The concept of using a fixed exponent of 0.75 for the prediction of clearance does not seem to be appropriate. From the data published by Mahmood and Balian [10], it can be seen that the exponents of allometry range from 0.35 to 1.39. The mean of the exponents is 0.78, which is close to 0.75, but given the wide range of exponents, it is obvious that using a fixed exponent of 0.75 will produce serious errors in the prediction of clearance for many drugs. However, it should be noted that the use of fixed exponent may be helpful when pharmacokinetic data from only one species are available. This approach may provide a rough estimate of clearance but the probability of a large error in the prediction of clearance is fairly high.

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Incorporation of in vitro Data in in vivo Clearance

Human liver microsomes contain different cytochrome P450 (CYP450) isozymes which are responsible for the biotransformation of xenobiotics and endogenous substances. With the understanding of the role of cytochrome P450 in the biotransformation of drugs, it is possible to characterize the metabolic pattern of a drug. Analysis of the literature indicates that there are several isozymes (CYP3A4, CYP2D6, CYP2C9, CYP1A2, CYP2C19) which are responsible for drug metabolism [13]. There are, however, three major isozymes (CYP3A4, CYP2D6, CYP2C9) which are responsible for the metabolism of almost 90% of drugs [13].

Characterization of drug metabolism in in vitro and extrapolation to in vivo is gaining momentum. In order to improve the prediction of clearance in humans, incorporation of in vitro clearance in in vivo clearance has been proposed. Houston [14] has published a comprehensive review article on this topic. Lave et al. [15] examined several methods (simple allometry, product of clearance and brain weight, and in vitro-in vivo method) to predict clearance of 10 drugs that are mainly eliminated through hepatic metabolism. In their approach, the authors determined the rates of metabolism of these drugs in various animal species and human liver microsomes and hepatocytes. Using the in vitro metabolism data and combining it with the in vivo data from animals, they predicted the in vivo clearance in humans using allometric scaling techniques. The in vivo clearance of each species was normalized by in vitro clearance as follows:

$$\frac{CL_{\text{in vivo animal}} \times CL_{\text{in human hepatocytes or microsomes}}}{CL_{\text{in animal hepatocytes or microsomes}}} \tag{8}$$

Lave et al. [15] concluded that integrating the in vitro data from the allometric approach with data obtained from at least three animal species improved the predictions of human clearance as compared to the approach of simple allometry.

Mahmood [16] reanalyzed Lave's data [15] and concluded that the normalization of clearance by MLP (as required based on the exponents) could have produced the same results as observed when in vitro clearance was incorporated in in vivo clearance. In the reanalysis of Lave's data, the approach of product of brain weight and clearance could not be applied as the exponents of the simple allometry were less than 1.

In a separate study, Obach et al. [17] used 12 different methods for the prediction of clearance and concluded that in vitro approach was the best method for the prediction of clearance. On average the predicted clearance was within 70–80% of actual values. The authors, however, compared the

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predicted clearance of the studied drugs using simple allomtery or MLP randomly.

Indeed, the in vitro approach is one of many attempts to improve the predictive performance of allometry for the prediction of clearance. However, the method has not been thoroughly tested and there are very few published data. Furthermore, the limitations of in vitro approach should be kept in mind. A definitive disadvantage of in vitro approach is the necessity of measuring in vitro clearance. The approach cannot be applied to those drugs which are renally excreted. Therefore, at this time caution and sound scientific judgement should be used to assess the reliability of the predicted clearance by in vitro approach. Extensive work will be needed in this direction before establishing the advantage and accuracy of in vitro approach in predicting clearance of drugs over other existing methods.

Number and Suitability of Species for the Prediction of CL

Since testing several species will add time and cost of drug development, it is always desirable to know the minimum number of species which can provide a reasonable accurate estimation of pharmacokinetic parameters in humans for a given drug. Mahmood and Balian [18] investigated whether clearance in humans can be predicted using two species as accurately as that of the predictions obtained by using three or more species (excluding human). Based on the evaluation of 12 compounds the authors concluded that three or more species are needed for a reliable prediction of clearance.

Campbell [19] investigated the suitability of a particular species for the prediction of clearance in humans. He reported that the prediction of clearance in humans was best predicted when data from rhesus or cynomolgus monkey were used with MLP. The rat was the next best species for the prediction of human clearance whereas dog appeared to be a poor predictor of clearance in humans. Based on limited data analysis, the author noted that pig also may be a poor predictor of clearance in humans, especially when MLP is incorporated in the scaling.

Role of Protein Binding for the Prediction of Clearance

Drug-protein binding is a reversible process and drugs may bind to albumin (weak acidic drugs) and alpha-acid glycoprotein (weak basic drugs). Drug-protein binding is influenced by a number of factors such as physicochemical propoerties of drug, concentration of drug as well as concentration of protein present in the body, the affinity between drug and protein and disease states such as hepatic or renal impairment.

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The kinetics of drug-protein binding can be described by the law of mass action by the followng equation:

Protein (P)+Drug (D)→Drug-Protein Complex (PD)

An association binding constant (Ka) between drug molecule and protein can be given as follows:

$$\mathrm{Ka} = \frac{(\mathrm{PD})}{(\mathrm{P})(\mathrm{D})} \tag{9}$$

The extent of drug-protein complex formed is dependent on Ka. Drugs strongly bound to proteins have a large Ka values. The number of binding sites *(n)* and the association constant (Ka) can be determined by the following equation:

$$r = \frac{n\mathrm{ka}(\mathrm{D})}{1 + \mathrm{Ka}(\mathrm{D})} \tag{10}$$

where n is the number of the binding sites per protein molecule and r is the moles of drug bound per mole of protein.

A double reciprocal plot of $1/r$ vs. free drug concentration (1/D) yields a straight line whose intercept is $1/n$ and the slope is $1/n$ka.

Another graphical technique known as scatchard plot can also provide binding constants and binding sites. A plot of r/D vs. r yields a straight line whose intercept is $n$ka and slope is -Ka.

Plasma protein binding vary considerably among animal species which in turn can influence the distribution and elimination of drugs. Due to this variability of plasma protein binding among species, it appears logical to predict unbound clearance in humans from animals. The unbound intrinsic clearance of many drugs such as antipyrine [8], phenytoin [20], clonazepam [20], caffeine [21], and cyclosporine [22] with or without normalization to MLP has been reported in the literature. However, a systematic comparative study (with the exception of two recent studies) to evaluate if indeed unbound clearance can be predicted with more accuracy than total clearance is lacking. Despite this lack of comparative study, it is widely believed that unbound clearance can be predicted with better accuracy than total clearance.

Obach et al. [17] in a comparative study attempted to predict the clearance of several drugs with or without taking protein binding into consideration. Based on average-fold error (1.91 without protein binding and 1.79 with protein binding), a slightly improved prediction of unbound

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TABLE 2 Observed and Total Predicted Clearance (mL/min) of Several Drugs with or without Considering Protein Binding

Drug	Observed total CL	Predicted total CL	Observed unbound CL	Predicted unbound CL	Predicted total CL*
Diazepam	26	861	813	25,525	817
Defetilide	331	312	836	717	258
Sildenafil	420	568	10,500	3,348	134
Tamsulosin	48	814	4,800	10,218	102
GV150526	5–7	320	3,000	2,554	5
Cyclosporin	273	716	4,200	8,731	611
Cefoperazone	71	76	402	332	58
CI-921	188	89	170,000	33,000	37
Morphine	1,680	3,505	2,585	6,331	4,115
Quinidine	330	1,452	2,538	18,639	2,423

*Obtained by multiplying the predicted unbound clearance in humans by free fraction of drug in human plasma. For example, the predicted unbound clearance of tamsulosin in humans was 10, 218 mL/min and f_u was 0.01. Therefore, the predicted total clearance in humans was 10, 218×0.01=102 mL/min.

clearance was noted, though for all practical purposes this difference may not be of any significance.

Mahmood [23] using the rule of exponents compared the total and unbound clearance of a wide variety of drugs to determine whether unbound clearance of a drug can be predicted more accurately than total clearance, and if there is any real advantage of predicting unbound clearance. The results of the study indicated whether a drug is excreted renally or by extensive metabolism, unbound clearance may or may not be predicted any better than total clearance. In his analysis, Mahmood noted that there are drugs whose unbound clearance can be predicted better than total clearance or vice versa, but at this time it is not possible to determine a priori for which drug unbound or total clearance can be predicted better. Overall, Mahmood's analysis indicated that correction for protein binding (unbound clearance) may or may not be helpful for the improved prediction of clearance in humans from animal data (Table 2).

Prediction of Clearance for Renally Secreted Drugs

Besides hepatic metabolism, drugs can also be cleared by renal route. Renal clearance is the sum of three processes: glomerular filtration, tubular secretion, and tubular reabsorption. As a general rule of thumb, renal

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clearance greater than 130 mL/min indicates that the secretion mechanism is involved, whereas a renal clearance less than 130 mL/min indicates tubular reabsorption. No matter what is the renal clearance of a drug it is possible that filtration, secretion, and reabsorption processes are simultenously in operation.

Tubular secretion is an active transport process and is independent of plasma protein binding but dependent on renal blood flow [24]. Drug secretion also depends on the affinity of the drug for carrier proteins in the proximal tubule, the rate of transport across the tubular membrane, and the rate of delivery of the drug to the site of secretion [24]. All these factors can be described by following equation:

$$CL_S = \frac{RBF \times f_b \times CL_i}{RBF + f_b \times CL_i} \tag{11}$$

where RBF is renal blood flow, f_b is free fraction of drug in blood, and CL_i is intrinsic secretion clearance.

Interspecies scaling of drugs for the prediction of clearance may become complicated due to the differences in the mechanism of excretion of drugs in different species. It is possible that a drug is extensively secreted in animals but in humans either drug is not secreted or secretion plays a minor role in the elimination of drug or vice versa. Mahmood [25], using 10 renally secreted drugs, demonstrated that it is likely that the predicted total and renal clearances for renally secreted drugs may be lower in humans than the observed clearances. The exponents of total clearance of 10 studied drugs ranged from 0.581 to 0.930. In this study, the predicted total clearance of seven out of ten drugs was lower by 11–65%. Mahmood and Balian's proposed rule of exponents did not help to improve the prediction of total clearance for these drugs. The predicted renal clearance also did not follow any particular trend, i.e., for some drugs the predicted clearance was higher than the observed clearance or vice versa. The prediction of renal clearance was improved by normalizing the renal clearance by a "correction factor" for animals which exhibited renal secretion. The "correction factor" was obtained by the following equation:

$$\frac{(\text{Glomerular filtration rate} \times \text{Kidney blood flow})}{(\text{Body weight} \times \text{Kidney weight})} \tag{12}$$

The concept of a "correction factor" is based on the fact that renal secretion of drugs is based on blood flow. Since the size of the kidneys, body weight, kidney blood flow, and glomerular filtration rate (GFR) vary from species to species and can be related by allometry, a correction factor as described in

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Eq. (12) was found to be suitable in order to improve the prediction of renal clearance.

Though the proposed approach for the prediction of renal clearance for renally secreted drugs worked fairly well on the tested drugs, due to small sample size of drugs used in this evaluation (n=10), more work will be needed in this direction. Since total clearance of renally secreted drugs could not be predicted with reasonable accuracy, a method which can improve the prediction of total clearance for such drugs requires investigation.

Selection of a First-time Dose in Humans Based on Predicted Clearance

Allometric scaling of a drug in development was performed using oral clearance of mouse, rat, guinea pig, monkey, and dog. Since the exponent of the simple allometry was 0.92, MLP approach was considered suitable for the prediction of clearance in humans. The predicted clearance was 1000 mL/min and 382 mL/min, using simple allometry and the MLP approach, respectively. Based on the prediction of clearance in humans, an initial dose of 200 mg was suggested. The human study, however, was initiated with 15 mg dose. Later, with dose escalation, it was found that the mean clearance of drug was between 350 and 400 mL/min following 250 and 500 mg dose, respectively, which was very close to the predicted values.

The above example clearly indicates that allometry can be very useful for the selection of a first-time dose to humans. In this example, the selection of a 15-times lower dose to iniate the study was not cost and time effective.

Volume of Distribution

There are three kinds of volumes which are frequently used in the interspecies scaling.

(a) The volume of distribution of the central compartment (V_c) is used to relate plasma concentration at time zero (C_0) of a drug and the amount of drug (X) in the body [26]

$$X=V_C \times C_0 \tag{13}$$

A small V_c (<3 L) indicates that most of the drug is in the plasma, whereas a large V_c (>7 L) indicates that the drug has concentrated in the extra vascular space.

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(b) The volume of distribution at steady state (V_{ss}) can be estimated from the following equation:

$$V_{SS} = \frac{\text{Dose} \times \text{AUMC}}{\text{AUC}^2} = \text{CL} \times \text{MRT} \tag{14}$$

where MRT is mean residence time=AUMC/AUC (15)

and AUC and AUMC are area under the curve and area under the momemt curve, respectively.

(c) The volume of distribution by area (V_{area}) also known as V_b can be obtained from the following equation:

$$V_b = \frac{\text{Clearance}}{b} \tag{16}$$

where b is elimination rate constant.

Physiological factors such as plasma protein, tissue binding, total body water and binding to erythrocytes may effect the distribution of drugs in the body. Therefore, a drug in the body can be accounted for inside plasma and outside plasma. The following equation can describe the relationship:

$$V = V_p + V_t\left(\frac{f_{up}}{f_{ut}}\right) \tag{17}$$

where V_p is plasma volume, V_t is tissue volume, and f_{up} and f_{ut} are the fraction of unbound drug in plasma and tissue, respectively. Drugs extensively bound to plasma proteins ($f_{up} << f_{ut}$) will have small volume of distribution.

In an attempt to establish relationship between binding to plasma proteins and volume of distribution of drugs in animals and man, Swada et al. [27] investigated the relationship between the volume of distribution (V_{ss}) and plasma protein binding of b-lactams. Swada et al. [28] also investigated the relationship between the unbound volume of distribution of tissues (V_t/f_{ut}) and f_u (fraction unbound) of nine acidic and six basic drugs in the rat and in humans. The authors concluded that there was little difference in V_t/f_{ut} of basic drugs between animals and man and that volume in man from animal data was predicted with more accuracy using V_t/f_{ut} than using volume against f_u.

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Obach et al. [17] used four different methods to predict V_{SS}, and based on their geometric mean, prediction accuracy concluded that unbound V_{SS} can be predicted better than the total V_{SS}.

Conceptually there should be a good correlation between body weight and volume among species and indeed this is the case. Generally the exponents of volume are around 1.0, which indicates that body weight and volume are directly proportional. However, this may not be the case for all drugs, and exponent as low as 0.58 (diazepoxide [29]) has been noted. Overall, volume of distribution can be predicted in humans from animals with reasonable accuracy. As noted by Mahmood and Balian [18], unlike clearance, volume can be predicted in humans with fair degree of accuracy using two species.

Though literature indicates that V_c, V_{SS}, or V_b are predicted indiscriminately in humans from animals, it has been shown by Mahmood [30] that V_c can be predicted with more accuracy than V_{SS} or V_b. In fact V_{SS} or V_b may not be of any real significance for the first-time dosing in humans and can be estimated from human data.

V_c can play an important role in establishing the safety or toxicity for the first-time dosing in humans. Since an administered dose is always known, the predicted V_c can be used to calculate plasma concentration of a drug at time zero (C_0) following intravenous administration. This initial plasma concentration may provide an index of safety or toxicity. Furthermore, V_c can also be used to predict half-life, if clearance is known ($t_{1/2}$=0.693 Vc/CL).

Elimination Half-life and Mean Residence Time

It is difficult to establish a relationship between body weight and half-life ($t_{1/2}$) since half-life is not directly related to the physiological function of the body rather it is a hybrid parameter. A poor correlation between $t_{1/2}$ and body weight across the species may give a poor prediction of half-life. Like clearance, the allometric exponents of half-life using body weight widely varies. In his evaluation of 18 drugs, Mahmood [30] reported that the exponents of half-life of these drugs varied from 0.066 to 0.547.

Due to the difficulty in estabishing an allometric relationship between body weight and half-life, some indirect approaches for the estimation of half-life have been suggested.

Bachmann [12], Mahmood and Balian [9], and Obach et al. [17] used the following equation to predict the half-lives of many drugs.

$$t_{1/2} = \frac{0.693V}{CL} \tag{18}$$

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Though this approach has been found to be suitable for the prediction of half-life for many drugs in humans, it is also necessary that one must predict both CL and volume in humans with reasonable accuracy.

Another indirect approach to predict half-life was suggested by Mahmood [30]. In this approach, first mean residence time (MRT) was predicted and then the predicted MRT was used to predict half-life in humans using the following equation:

$$t_{1/2} = \frac{\text{MRT}}{1.44} \tag{19}$$

The results of this study indicated that MRT can be predicted in humans with fair degree of accuracy from animal data. The exponents of MRT of the studied drugs varied from -0.260 to 0.385 (Table 3). The indirect estimation of half-life using MRT was fairly close to the observed values (Table 3).

TABLE 3 Predicted vs. Observed MRT and Predicted Half-life from MRT in Humans from Animal Data

Drugs	Exponent	*R*	Obs. MRT	Pred. MRT	Obs. T1/2	T1/2 from MRT
Tolcapone	0.283	0.875	1.20	1.70	1.30	1.20
Lamifiban	0.385	0.915	2.50	4.10	2.10	2.90
Interferon	0.231	0.776	2.40	2.40	5.10	1.63
AZT	0.107	0.827	0.90	1.14	1.10	0.80
Erythromycin	0.137	0.883	2.10	3.20	2.20	2.20
Oleandomycin	0.123	0.851	1.40	3.10	1.00	2.10
Amascrine	0.381	0.975	6.00	8.20	4.70	5.70
CI-921	0.228	0.682	2.00	3.80	2.60	2.60
Sematilide	0.186	0.946	3.10	3.60	2.70	2.50
Sch-34343	0.317	0.963	0.73	0.83	1.00	0.60
Ciprofloxacin	0.037	0.132	4.80	2.95	4.30	2.05
Cyclosporine	−0.260	0.337	5.50	2.90	6.20	2.00
Cefotetan	0.339	0.906	4.40	2.10	3.40	1.50
Cefmetazole	0.220	0.886	1.28	0.87	0.90	0.60
Cefoperazone	0.351	0.853	2.30	2.50	2.35	1.80
Moxalactam	0.289	0.999	2.40	1.70	1.30	1.70
Cefpiramide	0.373	0.917	6.70	4.50	5.00	3.10
Cefazoline	0.241	0.914	2.00	1.75	1.50	1.20

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Though Eqs (18) and (19) are only true for one compartment model, both these equations may also be used in a multicompartment system for prediction purposes.

Species-Invariant Time Methods

In chronological time there is an inverse relationship between the size of the animal and the heart beat and respiratory rates, in other words, as the size of the animals increases their heart beat and respiratory rates decrease. On the other hand, on a physiological time scale, regardless of their size all mammals have the same number of heart beats and breaths in their lifetime. Therefore, one may define physiological time as the time required to complete a species-independent physiological event. Thus in smaller animals the physiological processes are faster and the life span is shorter.

Chronological time, also known as species-invariant time, can be transformed into physiological time. Dedrick et al. [31] were first to use the concept of species-invariant time when they used the pharmacokinetic parameters of methotrexate in five mammalian species following intravenous administration as an example. The chronological time was transformed into physiological time using the following equations:

$$Y\text{-axis} = \frac{\text{concentration}}{(\text{Dose}/\text{W})} \tag{20}$$

$$X\text{-axis} = \frac{\text{time}}{\text{W}^{0.25}} \tag{21}$$

where W is the body weight.

By transforming the chronological time to physiological time, Dedrick and co-workers demonstrated that the plasma concentrations of methotrexate were superimposable in all species. They termed this transformation as equivalent time.

Later, Boxenbaum [20] introduced two new units of pharmacokinetic time, kallynochrons, and apolysichrons. Kallynochrons and apolysichrons are transformed time units in elementry Dedrick plot and complex Dedrick plot, respectively.

Kallynochrons (elementry Dedrick plot):

$$Y\text{-axis} = \frac{\text{concentration}}{(\text{Dose}/\text{W})} \tag{22}$$

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$$X\text{-axis} = \frac{\text{time}}{W^{1-b}} \tag{23}$$

where b is the exponent of clearance.

Apolysichrons (complex Dedrick plot):

$$Y\text{-axis} = \frac{\text{concentration}}{(\text{Dose}/W^{c})} \tag{24}$$

$$X\text{-axis} = \frac{\text{time}}{W^{c-b}} \tag{25}$$

where b and c are the exponents of clearance and volume, respectively.

Dienetichrons

Boxenbaum [20] introduced a new time unit known as dienetichrons by incorporating the concept of MLP in physiological time. The transformation of chronological time to dienetichrons can be obtained by dividing the X-axis or time by MLP. For example, for elementry Dedrick plot, X-axis or time was normalized as follows:

$$X\text{-axis} = \frac{\text{time}}{\text{MLP}} \times \frac{1}{W^{1-b}} \tag{26}$$

Though some investigators [32–34] have used the concept of species-invariant time in their allometric analysis, a direct comparison of allometric approaches with species-invariant time has not been systematically evaluated.

In a study, Mahmood and Yuan [35] compared the empirical allometric approaches with species-invariant time methods using equivalent time, kallynochron, apolysichron, and dienetichrons. Clearance, volume of distribution, and elimination half-life of three drugs (ethosuximide, cyclosporine, and ciprofloxacin) were compared using allometric approach and species invariant time methods. Overall, the species invariant time method did not provide any improvement over conventional allometric approach. Especially, the equivalent time approach did not predict plasma concentrations or pharmacokinetic parameters as accurately as elementry or complex Dedrick plots. This may be due to the fact that equivalent time approach uses a fixed exponent of 0.25 for elimination half-life. It should be noted, however, that the exponent of half-life of drugs is not always 0.25 [30]. The exponents of half-life for ethosuximide, cyclosporine, and ciprofloxacin in this study were 0.47, -0.24, and 0.04, respectively.

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Normalization of clearance by MLP provided substantial improvement in the prediction of clearance for cyclosporine and ciprofloxacin (according to "rule of exponents" as the exponent of simple allometry was greater than 0.7). The incorporation of MLP in the species invariant time method substantially underpredicted the clearance and overpredicted the half-life by more than 20-fold. It was noted by the authors that this inaccurate prediction of clearance and half-life was mainly due to the prolonged sampling times in humans following the normalization of MLP. This increased the AUC and prolonged the half-life of cyclosporin and ciprofloxacin.

The findings of this study were based on the limited number of drugs (n=3). Overall, the results of this study indicated that both simple allometry and species invariant time methods would give almost similar results. Species invariant time method may be helpful in gaining some insight about plasma concentrations of a drug but the accuracy of this method in predicting plasma concentrations in man may not be reliable.

Prediction of Pharmacokinetic Parameters Using Pharmacokinetic Constants

Besides Species invariant time method, pharmacokinetic constants have been also used by some investigators to predict plasma concentrations in humans from animals.

The following equation represents a two-compartment model following intravenous administration.

$$C=Ae^{-at}+Be^{-bt} \tag{27}$$

where A and B are the intercepts on Y-axis of plasma concentration vs. time plot and a and b are the rate constants for the distribution and the elimination phases, respectively.

Equation [27] can be used to predict plasma concentrations as well as pharmacokinetic parameters (using predicted concentrations) in humans from animal data. Swabb and Bonner [36] and Mordenti [37] predicted plasma concentrations of aztreonam (one compartment model) and ceftizoxime (two compartment model) in humans from animal data using pharmacokinetic constants. Though Swabb and Bonner and Mordenti successfully used pharmacokinetic constants approach for the prediction of plasma concentrations and pharmacokinetic parameters, the suitability of this approach for the prediction of pharmacokinetic parameters in humans from animal data has not been thoroughly investigated.

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Mahmood [38] compared the predicted pharmacokinetic parameters of six drugs using either pharmacokinetic constants or conventional allometric approach. No trend in correlation between body weight and A, B, or *a* was found. For some drugs a good correlation between body weight and these parameters was obtained whereas a poor correlation was observed for other drugs. Though the predicted values of A and B were occasionally close to the observed values, the predicted *a* values were many folds higher or lower than the observed values which had substantial effect on the predicted plasma concentrations. Overall, the use of pharmacokinetic constants to predict pharmacokinetic parameters in humans from animal data did not provide any improvement over conventional allometric approach. Like species invariant time method, pharmacokinetic constant approach may provide some information about plasma concentrations of a drug but the accuracy of the method for the prediction of plasma concentrations in man may be questionable.

Absorption and Absolute Bioavailability

Prediction of absolute bioavailability in humans from animals due to the differences in the anatomical and physiological features of the gastrointestinal tract, dietry habits, blood flow through the gut and the liver, and the enzymatic activity of the metabolizing enzymes, is a complex task. Some animal models may provide a rough estimate of absolute bioavailability in humans and such rough estimates can also be of significant importance to identify problems of absorption and intestinal and hepatic metabolism in man.

Conceptually it is difficult to justify an allometric relationship between body weight and absolute bioavailability. Mahmood [39] using direct (body weight vs. absolute bioavailability) and several indirect approaches attempted to predict absolute bioavailability in humans from animal data. Five different methods were used to predict absolute bioavailability in humans:

i. body weight vs. absolute bioavailability (allometric approach)
ii. F=CL(IV)/CL(oral) (28)
iii. F=1-(CL(IV)/Q) (29)
iv. F=1-(CL(oral)/Q) (30)
v. F=Q/(Q+CL(oral)) (31)

where Q is hepatic blood flow (1500 mL/min). Methods II-V are indirect approaches. Fifteen drugs were used in this analysis. In Table 4 the

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TABLE 4 Predicted and Observed Absolute Bioavailability of 15 Drugs using Different Methods

		Predicted F (%)						
Drugs	Obs F (%)	Method I	Method II	Method III	Method IV	Method V	Range	Mid point
Actisomide	30–43	135	76	65	53	68	53–76	64
Amlodipine	63	86	86	78	75	80	75–86	80
Candoxatrilat	32	15	56	87	76	81	15–87	51
CI-1007	4	12	4	31	NA	5	5–31	18
Dofetalide	83	66	72	81	74	79	66–81	74
Faradifiban	24	5	15	94	60	71	5–94	50
Meloxicam	95	89	89	99	99	99	89–99	94
Metoprolol	38	53	53	45	NA	50	45–53	49
Morphine	24	9	16	32	NA	19	9–32	20
Nicardipine	7	9	9	47	NA	15	9–15	12
Recainam	67	132	130	69	76	81	69–81	75
Remikiren	0.3	0.3	0.3	41*	NA	0.7	0.3–0.7*	0.50
Sildenafil	35	70	67	65	48	66	48–70	59
Tacrolimus	25	8	5	NA	NA	3	3–8	5
Troglitazone	43–53	53	23	88	47	15	23–88	55
% **MAE**		**59**	**46**	**58**	**NC**	**49**		**33**

NC=Not calculated because there were only nine drugs available for this method.
NA=Not available. Oral clearance was greater than the liver blood flow (1,500mL/min).
*Method III not included in the analysis. Method I=body weight vs. absolute bioavailability; Method II: F-CL(IV)/CL(oral); Method III: F=1-(CL(IV)/Q); Method V: F=Q/(Q+CL(oral)).
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correlation coefficient between body weight and absolute bioavailability, exponents of allometric equation, and the predicted absolute bioavailability in humans from animals have been shown. Though for some drugs a good correlation between body weight and absolute bioavailability has been obtained, there is uncertainty in the prediction of absolute bioavailability in humans from animals. Overall, the results of the study indicated that all the five approaches predict absolute bioavailability with different degrees of accuracy and are unreliable for the accurate prediction of absolute bioavailability in humans from animals. Despite uncertainty in the prediction of absolute bioavailability in humans, the approach may provide a rough estimate of absolute bioavailability.

Sietsema [40] plotted absolute bioavailability in man against those in rodents, dogs, and monkeys. The correlation coefficient (r^2) for absolute bioavailability between man and rodent, man and dog, and man and primates was 0.4, 0.3, and 0.2, respectively. This poor correlation indicates that absolute bioavailability data in animals may be of moderate use for the prediction of absolute bioavailability in humans.

In recent years, attempts have also been made to correlate fraction of oral dose between rat and humans [41]. For the prediction of absorption in humans, methods such as intestinal permeability in rats [42, 43], jejunal permeability in humans [42, 43], and caco-2 cell permeability [44] have been proposed.

Prediction of Maximum Tolerated Dose (MTD)

In phase I clinical trials, not only the selection of the first dose to be administered to the patients is a challenge but also the issue of dose escalation is a complex task. A conservative low-dose approach will result in subtherapcutic or ineffective dose. On the other hand an aggressive dose escalation may result in producing toxicity. Certain class of drugs, for example anticancer drugs, are so toxic that for ethical reasons they can not be given to healthy subjects. Therefore, predicting MTD in humans from animal data may prove to be highly beneficial. For anticancer agents, generally 1/10 of the LD_{10} in mice or 1/3 of the toxic dose level (TDL) in the dog in mg/m^2 is used as the starting dose in phase I clinical trials [45]. Goldsmith et al. [46] reported that the use of 1/3 of the TDL would have produced significant toxicity in the patients for 5 out of 30 drugs. The authors further concluded that for a safe starting dose in phase I clinical trials, not only toxicology data from dog and monkey, but also data from rat, mice, and tumor-bearing mice should be included. Similary Homan [47] concluded that there was a 5.9% probability of exceeding the human maximum tolerated dose (MTD) if the starting dose in clinical trials were 1/3 of the TDL of large animal species (dog or monkeys). Rozencwig et al. [45]

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concluded that 1/6 LD_{10} in the mouse and 1/3 toxic dose low in the dog corresponds to an acceptable dose in humans provided both preclinical and clinical data are obtained under identical schedule and compared on a mg/m^2 basis. Mice and dogs may provide different informations for a given drug but combining data from both species can be helpful in determining the starting dose in humans for phase I clinical trials [45].

Mahmood [48] using 25 anticancer drugs examined whether or not MTD can be predicted from animals to humans. The predictive performance of two different approaches of allometry for the prediction of MTD was compared in humans from animal data. The two approaches to predict MTD in humans were: (i) the use of a fixed exponent of 0.75 and the LD_{10} in mice; and (ii) the use of LD_{10} (in case of mice) or MTD data from at least three animal species (interspecies scaling). The results of the study indicated that MTD can be predicted more accurately using interspecies scaling than using a fixed exponent of 0.75. Like clearance, it was noted that incorporation of mean life-span potential (MLP) can also be used to improve the prediction of MTD for some drugs. One-third of the predicted MTD from interspecies scaling can be used as a starting dose in humans. This approach may save time and avoid many unnecessary steps to attain MTD in humans.

Prediction of Inhalational Anesthetic Potency Minimum Alveolar Concentration (MAC)

Interspecies scaling is frequently performed to predict pharmacokinetic parameters from animals to man and a fair amount of research has been successfully conducted to correlate body weight with the pharmacokinetic parameter(s) of interest [5, 49, 50]. However, very little information is available for the prediction of pharmacodynamic parameters from animals to man. Travis and Bowers [51] applied the principles of allometry to the minimum alveolar concentration of several inhalational anesthetics. The authors found that not only there was a poor correlation between body weight of animals and the MAC but the slope of the allometry was statistically not different from zero. Lack of correlation between body weight and MAC and a slope nearly zero made it almost impossible to predict MAC in humans.

MAC is defined as the minimum concentration of inhalational anesthetic agent in the alveolus at steady state which will inhibit a muscular response to stimulus in 50% of patient population and is expressed as volume percent required at one atmosphere [52]. Thus MAC represents EC_{50} on a conventional quantal dose response curve.

Using a correction factor, Mahmood [53] attempted to predict MAC from animals to humans. The MAC values of 10 anesthetics were obtained

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from the literature. At least three animal species (excluding humans) were used in the scaling. Interspecies scaling of MAC was performed using the following two methods:

i. Using traditional allometric approach, the MAC of each drug was plotted against the body weight of the species on a log-log scale and from the resultant equation MAC was predicted in humans;
ii. MAC in each species was multiplied by a "correction factor" obtained by adjusting the lung weight of the species based on per kg body weight. The product of correction factor and the MAC was then plotted against body weight on a log-log scale.

Using the simple allometric approach, the correlation between body weight of the species and the MAC was found to be poor. The exponents of the simple allometry varied from -0.026 to 0.105. The mean of the exponents of all 10 drugs was 0.027 which was statistically not different from zero. The error of predicted values ranged from 28–134%. The predicted MAC in humans was overestimated at least by 50% for six drugs.

On the other hand, incorporation of "correction factor" substantially improved the correlation between body weight and the MAC. The exponents of the allometry varied from 0.078 to 0.218. The mean of the exponents of all 10 drugs was 0.127 which was statistically different from zero. The error of predicted values ranged from 2–92%. The predicted MAC in humans was overestimated by 50% for only two drugs.

It is difficult to visualize that there will be a correlation between body weight and MAC, since a change in a pharmacodynamic parameter may not simply be a function of change in body weight. The concept of a "correction factor" for anesthetic gases and vapors is based on the fact that these anesthtics are administered to patients at appropriate inspired concentrations. Depth of anesthesia is determined by the concentration of anesthetic agent in the brain. The rate at which an effective brain concentration can be acheived depends on the rates of transfer of inhaled anesthesia from the lung to the blood and from blood to the tissues, the solubility of the anesthetic from the lungs to the arterial blood, its concentration in the inspired air, pulmonary ventilation rate, pulmonary blood flow (change in cardiac output), and the partial pressure of the anesthetic between arterial and mixed venous blood. Considering all the abovementioned factors in order to achieve an adequate concentration of an inhaled anesthetic, it appears lung plays a vital role, as the lung is the site of drug delivery. Therefore, taking into account that the role of lung in maintaining an adequate concentration of a given anesthetic in the brain is vital and since the size of the lung and the body weight varies from species to

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species, normalization of the lung weight based on the body weight was found to be suitable for the improved prediction of MAC in humans from animals. Though data for inhaled drugs other than anesthetics were not evaluated, the findings of this study may be extended to other inhaled drugs. The concept of a correction factor for the prediction of a parameter of interest (especially for a pharmacodynamic parameter) for inhaled drugs other than anesthetics should be examined.

CONCLUSION

The allometric scaling of pharmacokinetic parameters can be useful to select a safe and tolerable dose for the first-time administration to humans. Thus scaling can provide a rational basis for the selection of a first dose in humans. Therefore, in recent years, interspecies scaling of pharmacokinetic parameters has drawn enormous attention. Over the years many approaches have been suggested to improve the predictive performance of allometric scaling. Though not perfect, these approaches are of considerable importance to understand and refine the concept of allometric scaling.

There may be anatomical similarities among species but there are external factors which will affect the allometric scaling. Experimental design, species, analytical errors, and physico-chemical properties of drugs such as renal secretion or biliary excretion may have impact on allometric extrapolation.

Despite the fact that allometry is empirical and occasionally fails to perform adequately, further investigation should be conducted to find the underlying reasons for failure.

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8

Analytical Method Validation

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INTRODUCTION

The purpose of this chapter is to describe the elements of analytical method validation promulgated by the U.S. Food and Drug Administration (FDA) for drug development, and to explain the reasoning for each component. This chapter is intended for individuals who are unfamiliar with analytical method validation, or new to drug development. Readers who are interested in more detailed experimental or statistical treatises of specific aspects of method validation are referred to elsewhere.

Analytical method validation is the process used to determine the capabilities and limitations of an assay. This process is very important because the data these assays generate are used to make chemical, pharmacokinetic, and pharmacodynamic conclusions about drugs. The ability to make these conclusions is of great importance, because they in turn are used to support claims regarding the safety and efficacy of new drugs to be used in human patients. This demonstration of safety and

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efficacy of a new drug or therapeutic is required by law in the United States, Europe, Canada, and Japan. Therefore, the failure to ensure the reliability of an analytical method, the data it generates, and the resulting conclusions can raise significant questions about the validity of the drug safety and efficacy claims.

Analytical method validation has been addressed by the U.S. Food and Drug Administration, the European Medicines Evaluation Agency (EMEA; the regulatory body of the European Union), and the regulatory agencies of other countries such as Australia, Canada, and Japan. As a result of the efforts of the International Committee on Harmonization (ICH), the differences in the approaches and requirements to analytical method validation by different countries have been minimized. However, the reader is cautioned that there may be different requirements in different countries and appropriate guidance should be sought for submissions elsewhere. The remainder of this chapter explains the characteristics of method validation that are promulgated by the U.S. FDA in the Guidance for Industry entitled "Bioanalytical Method Validation" [1]. The principles described in the document were established following a workshop cosponsored by the FDA and the American Association of Pharmaceutical Scientists (AAPS) in 1990 [2]. The workshop was attended by regulatory scientists, pharmaceutical industry scientists, and academicians involved in pharmaceutical analysis. The principles that were developed are described in the original draft Guidance for Industry, "Bioanalytical Method Validation for Human Studies" which is currently posted on the FDA internet site [3]. However, these analytical method validation principles were recently updated at a second workshop convened by the FDA and AAPS in January 2000 [4]. Additional guidance has been included for the newer analytical technologies of LC/MS/MS and ligand binding assays such as radioimmunoassays (RIAs) and enzyme-linked immuno-sorbent assays (ELISAs) [4].

Analytical method development and validation are usually completed prior to the start of preclinical and clinical pharmacology studies (bioavailability, bioequivalence, individual, or population pharmacokinetic studies) of new chemical entities intended for submission to the FDA as New Drug Applications (NDAs). Analytical method validation is also required for the development and assay of generic drugs, which are the subject of Abbreviated New Drug Applications (ANDAs), and veterinary drugs. Analytical method validations are also required for the Chemistry, Manufacturing and Controls (CMC) section of NDAs and ANDAs that describe the chemical quality and stability characteristics of the drug. However, the FDA Office of New Drug Chemistry issued a separate Guidance for Industry for the Validation of Chromatographic Methods, and the reader is referred to this document for specifics regarding CMC issues of NDAs and AND As [5–7].

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TYPES OF ANALYTICAL METHODS

Chromatographic methodologies have proved very useful for drug analysis. From the mid-1970s to the early 1990s, the most widely used analytical methodologies in drug development were gas-liquid (GC) and high performance liquid chromatography (HPLC).

Gas-Liquid Chromatography

In GC, samples are vaporized in the injection port, and sample constituents are then separated as they are moved along the length of the column by the carrier gas. Separation of the constituents is achieved because each compound possesses a characteristic rate of dissolution into the stationary phase and revolatilization into the mobile phase that is dependent upon the characteristics of the compound, and the stationary phase used in the method (see Fig. 1) [8]. The extent of separation can be increased or decreased to some extent by altering the temperature of the oven in which the chromatographic column is housed. Some advanced GC systems also incorporates hardware that allows for variable injection port temperatures to increase analyte separation. However, the main means of increasing the separation of the analyte from other sample constituents is the choice of the stationary phase/column used in the method. As each analyte exits the column, it is detected and quantified by a detector (e.g., mass spectrometer, electron capture, flame ionization detectors, etc.). Gas chromatography is generally characterized by great analytical sensitivity, often as low pg/ml, but it is limited by the need to volatilize the compounds of interest. Compounds with high boiling points are difficult to vaporize and cannot be quantified by GC very readily [8]. For this reason, HPLC has been more widely used.

HPLC

In HPLC, the samples are dissolved in a solvent and injected into the system. The analytes are then separated from other sample constituents by the differential rates of dissolution into the mobile phase and the stationary phase. The rate of this process is a characteristic of the analyte, mobile, and stationary phases used in the system. Increased or decreased separation can be obtained by altering the composition of the mobile phase solvent (i.e., changing the solvent polarity). Analytes are detected upon exiting the column by several types of detectors (i.e., UV-VIS, fluorescence, electrochemical, mass spectrometers, Fourier Transformed Infrared (FTIR) detectors). The main limitation with HPLC is the ability to dissolve the

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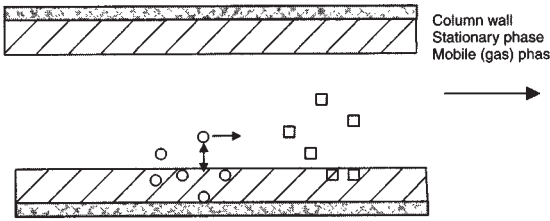

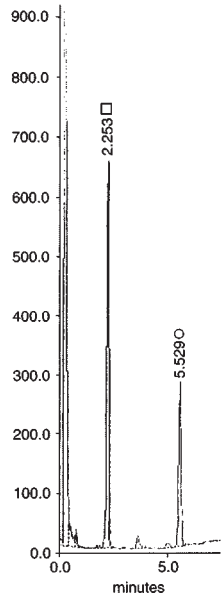

FIGURE 1 Chromatographic Separation. In GC, compounds are acted on by two forces: the carrier gas (mobile phase) which sweeps the molecules along the column (but does nothing to separate molecules), and dissolution of the compounds into the stationary phase. Separation is accomplished by the differences in the rate of dissolution of the molecules into and out of the stationary phase. The circles represent molecules with lower vapor pressures, which spend more time dissolved in the stationary phase. The circles are held up by the stationary phase, whereas the molecules represented by the squares have a higher vapor pressure (lower boiling point), and spend more time in the mobile phase, which sweeps these molecules out of the column faster than the circles. Therefore, the squares are swept through the column to the detector faster than the circles. (The squares have a shorter retention time.) In HPLC, these interactions are similar. The difference is that a solvent is used in the mobile phase, and it contributes to the forces that separate the molecules.

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sample in a solvent. This difficulty, however, is much less of a problem in HPLC than sample vaporization is in GC. The limit of detectability is usually lower with GC than HPLC (10 to 100 times), depending on the type of detector used. Generally, UV-VIS and fluorescence detectors in HPLC provide less sensitivity than GC detectors, but electrochemical and mass spectrometric detectors could provide equivalent sensitivity to GC systems.

LC/tandem Mass Spectrometry

Currently, the most widely used analytical technology is LC/MS/MS. Traditionally, these systems were cumbersome and difficult to use, but recent advances in technology and automation have made LC/MS/MS systems the stalwart of current analytical methodologies. LC/MS/MS depends on HPLC to separate the analyte from other matrix constituents as described in the preceding section, but the use of tandem mass spectrometry allows for the detection of very small quantities of drug, in addition to generating information about the chemical structure of the analyte which allows for analyte identification.

Ligand-Binding Assays

In addition to LC/MS/MS, greater use is currently made of nonchromatographic techniques. The two most prevalent techniques, radioimmunoassays (RIAs) and enzyme-linked immunosorbent assays (ELISAs) are ligandbinding techniques. These assays are based on specific or relatively specific antibodies that are developed for the analyte of interest (see Fig. 2).

RIAs

In a RIA, the analyte is incubated in a buffer with the antibody and a known quantity of radiolabeled analyte. After incubating these reactants for a period, the samples are centrifuged and the radioactivity in the bound, pellet fraction is counted (in some cases, the unbound tracer in the supernatant is counted instead). As the amount of analyte increases, more radioactive analyte is displaced and the amount of radioactivity in the pellet decreases. Therefore, low radioactivity corresponds to higher amounts of actual analyte in the sample (see Fig. 3).

ELISAs

In an ELISA, the antibody is usually bound to a surface, and linked to some type of enzymatic reporter system (for instance, horseradish peroxidase). Typically, the enzymatic reporter systems are linked to the surface of 96-well

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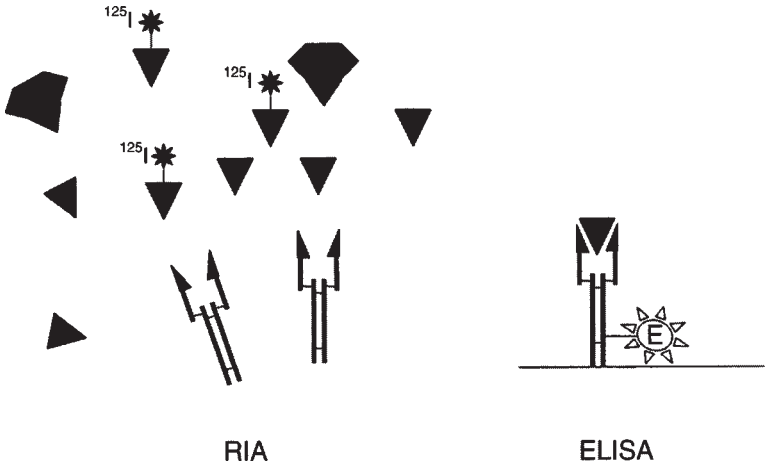

FIGURE 2 RIAs and ELISAs. These assays are ligand-based assays. The triangle represents the analyte of interest. In the RIA, the analyte displaces the binding of a known quantity of radiolabeled analyte (triangle with ^{125}I). The oddly shaped molecule with a triangular edge represents a potential interference, namely a molecule with a similar hapten as the analyte of interest. In the ELISA, once the analyte binds the antibody (which is bound to a surface), the enzyme linked to the antibody is activated to signal the interaction.

plates. Samples are added along with the necessary reactants, and gently mixed. After a defined period of incubation, the reaction in each well is "stopped" and the amount of analyte is quantified (often using a spectrophotmetric plate reader). One of the major drawbacks with ligandbased assays is antibody binding to nonanalyte entities. This type of binding will produce overestimates of the analyte quantity. It can be difficult to determine whether this process has occurred because unlike chromatography, there is no visual output to assess. Therefore, greater care has to be taken to ensure that no interference occurs in these types of assays.

ANALYTICAL METHOD VALIDATION

After choosing the best analytical method to be used, which includes the type of analytical principle (e.g., HPLC), hardware, extraction, and reconstitution procedures (isolation of the analyte from the sample matrix), the limitations of the complete assay need to be determined. Analytical method validation essentially consists of three discrete steps: (1) assessing

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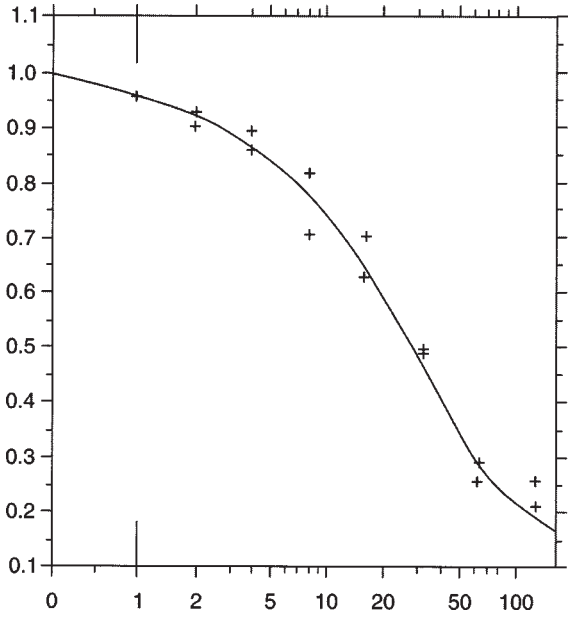

FIGURE 3 RIA Standard Curve. The X-axis is the log of the concentration range (1 to 100 units), and the Y-axis reports the amount of radioactive tracer that is bound to the antibody. As increasing amounts of nonlabeled analyte from the sample are incubated, increasing fractions of the radioactive tracer are displaced. Therefore, the curve declines with increasing concentrations of unlabeled analyte.

the limits of the analytical assay, (2) determining the effect of sample handling, and (3) monitoring assay quality during practical use.

Assessing the Limits of the Analytical Assay

Several aspects are assessed, and these are summarized in Fig. 4. Essentially, the bioanalyst needs to define a box that is bounded by the upper and lower limits of acceptable error, and the upper and lower limits of quantification. Once these limits are defined, we will be confident that experimental determinations of analyte concentrations that are within this box are reliable. The specific assay characteristics of interest are as follows.

The Standard Curve (Calibration Curve)

The relationship between drug concentration and the response of the analytical system needs to be determined. This mathematical relationship will allow us to later determine analyte concentrations of unknown clinical

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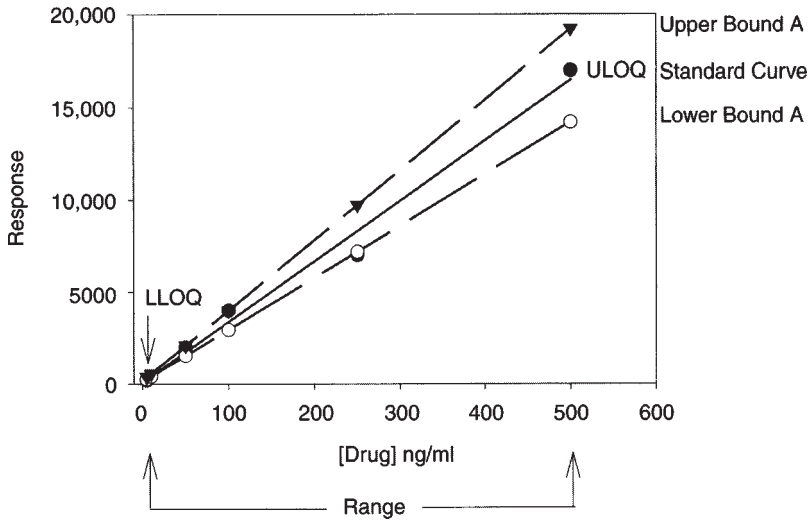

FIGURE 4 Standard Curve. The detector responses to a drug are plotted against six duplicate concentrations of drug ranging from 5 to 500ng/mL (•). The upper level of acceptable error in the drug concentrations is represented by the triangles, and the lower level by the open circles. The ULOQ is 500 ng/ml, and the LLOQ is 5 ng/ml. The solid line through the actual data was linearly regressed, and generated an equation for a straight line with the form $Y=AX+B$, where Y is the machine response, A is the slope of the curve, X is the drug concentration and B is the intercept on the y-axis. With the values of A and 8, the value Y for unknown samples is determined by analysis, and the corresponding concentration is then back-calculated.

samples from the response obtained from the analytical method. The standard curve of the method is specific for each drug in a specific matrix (e.g., blood, plasma, urine, cerebrospinal fluid, etc.). If the drug will be measured in plasma during the clinical study, the standard curve should be constructed by spiking drug into plasma, and then extracting and analyzing the concentrations. The use of different solvents such as water or methanol is not recommended because there may be differing solvent characteristics (such as solubility, protein binding, etc.), and this could complicate the interpretation of the data. The drug stock solution must be made in a solvent, but all subsequent dilutions should be in sample matrix.

If samples will be taken from more than one matrix (e.g., plasma and urine), then standard curves must be constructed for each. The same is also true if more than one analyte is to be measured (e.g., parent drug and metabolite). Although parent and metabolite may be simultaneously quantified from the same sample, a standard curve for each specific analyte

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must be constructed. It is also advisable to incorporate the use of an internal or external standard in sample preparation, although this step is not a requirement for method validation. Standard curves should be constructed with a minimum of six drug or analyte concentrations spiked in the appropriate matrix (see Fig. 4). Once these standards are measured, the data should be plotted (response vs. analyte concentration), and the simplest curve which best fits the data should be generated to describe the relationship. Zero or blank samples should not be included in the curvefitting procedure because the assay is characterized by a lower limit of quantification which is higher than "zero" or no drug, and inclusion of this point might alter the fit of the curve. Curves generated without weighting of the data are preferred, but weighting the data is permitted. Usually, weighting is used in cases where the range in drug concentrations spans several orders of magnitude, and weighting helps account for the heterocedasticity in the data. The relationship that is derived is then used to back-calculate drug concentrations from clinical study samples. The slope of the curve indicates the sensitivity of the assay; small changes in concentration that induce large changes in response indicate a sensitive method [9].

Range

The range of the standard curve should cover the expected range of concentrations that will be covered in the clinical study. The range is bracketed by the lower limit of quantification (LLOQ or LOQ, see Fig. 4; data below LLOQ are often reported as BQL—below quantification limit) and the upper limit of quantification (ULOQ, see Fig. 4). Extrapolation of drug concentrations beyond either limit is *not* acceptable. Concentrations below the LLOQ cannot be measured, unless further analytical development is conducted. One possible approach is validating the use of larger sample volumes at concentrations near the LLOQ [9]. Drug concentrations that are beyond the ULOQ of the assay should be diluted and reassayed. Determining the effect of sample dilution is helpful. Sample dilutions should be conducted using like matrix, e.g., plasma for plasma samples, urine for urine samples, etc. Use of a nonlike matrix can alter the physicochemical conditions acting on the analyte, causing nonlinearity which may lead to errors in sample quantification.

LLOQ

The LLOQ is the lowest concentration that can be reliably measured with the assay. The LLOQ is often confused with the lower limit of detection (LLOD; LOD). The LLOD is the lowest response that can be detected by

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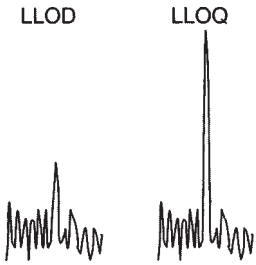

FIGURE 5 LLOQ. LLOD, is defined as two times the background noise. LLOQ is defined as five times the background noise.

the analytical hardware. It is usually defined as the signal that is two or three times higher than the background noise (signal to noise ratio of 2 or 3; see Fig. 5). LLOQ is often defined as some multiple of the LLOD (e.g., three or five times higher). However, the LLOQ is defined in the FDA Guidance as the response that is at a minimum of five times higher than the response to a blank sample, which is slightly ambiguous because it is not necessarily related to the minimum ability of the detector to measure a signal. The EMEA adopted the ICH definition, which defines the LLOQ as 10 times higher than background [10]. In Canada, the LOQ is deemed acceptable if the precision has been adequately demonstrated for that concentration.

Selectivity

The selectivity (also referred to as specificity) is the ability of the assay to measure the drug or analyte without interference from other constituents in the sample matrix. In chromatographic systems, selectivity is demonstrated by comparing the detector response in the presence of drug, to a blank sample of plasma that was not exposed to the analyte (see Fig. 6). Comparisons of the chromatograms, and the peak area or heights between the drug and the blanks are made to demonstrate selectivity. Blank chromatograms should be obtained from sample matrix (e.g., plasma) obtained from six different sources that have not been treated with the drug. Furthermore, it is also advisable to determine whether any medications to be co-administered during the clinical study will interfere with the quantification of the analyte of interest. In addition, if an internal standard is used in the method, blanks with internal standard should also be compared to the drug and completely blank matrix to demonstrate that the internal standard will not interfere with analyte quantification. For other nonchromatographic types of analytical methods, such as RIAs and

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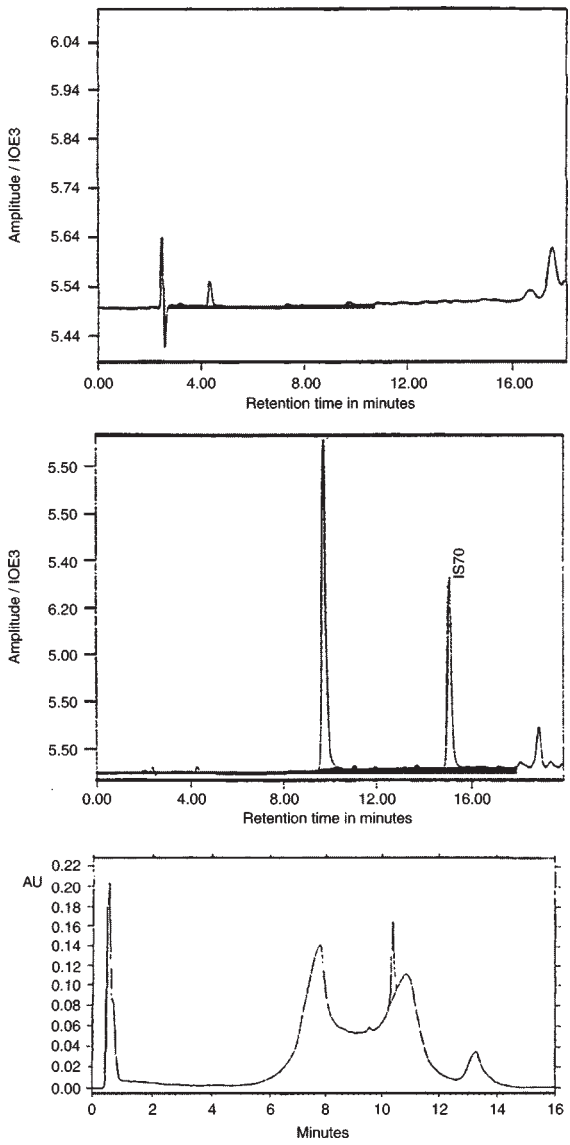

FIGURE 6 Selectivity. The upper curve is a HPLC chromatogram of blank plasma. In the middle tracing, drug X and an internal standard (ISTD) were spiked into plasma. In comparison with the blank plasma, it can be concluded that the assay provides good selectivity for this drug. The bottom chromatogram is an example of assay in which the peak of interest (retention time of 10 min) is interfered with by a larger unknown peak.

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ELISAs, the demonstration of selectivity is more difficult because there is no visual representation of the assay. In ligand-binding assays, an antibody binds to some chemical entity, and quantification is based on some radioactive tracer or enzyme activity. However, how does one know that the antibody does not bind some entity other than the analyte of interest? In these cases, the best assessment of selectivity is made by screening ligand crossreactivity with other compounds known to be chemically similar to the drug (i.e., endogenous compounds, drug fragments, etc.). The difficulty is there may be interactions with compounds that are not predictable. Therefore, the selectivity cannot be known absolutely with these methods. In these cases, it also recommended that selectivity of the ligand-based assays should be confirmed with the use of other analytical methods that rely on different principles (e.g., HPLC). In addition, nonspecific binding of the ligand may occur, and the prozone effect, i.e., nonspecific binding with buffer constituents, should also be assessed regularly [11].

Accuracy

The determination of accuracy indicates how close the measured concentration is to the true or nominal concentration (see Table 1). This step assesses the systematic error or bias of the entire analytical procedure (analyte extraction, reconstitution, analysis). Known amounts of analyte are added to the matrix and measured. A minimum of three concentrations that span the standard curve should be assessed, and at least five determinations or replicates should be conducted for each concentration. Accuracy is

TABLE 1 Intra- and Between-Run Accuracy and Precision of Drug X

		Nominal drug concentration (ng/mL)				
	Parameters ($n = 6$)	0.5	1.0	5.0	7.5	10.0
Intra-run	Mean	0.48	1.1	4.7	7.35	9.7
	S.D.	0.03	0.13	0.23	0.35	0.55
	Accuracy (%)	96	110	94	98	97
	Precision (% CV)	6.3	11.8	5.9	4.8	5.7
Between-run	Mean	0.42	0.89	4.3	6.5	9.3
	S.D.	0.09	0.12	0.48	0.91	0.92
	Accuracy (%)	84	89	86	87	93
	Precision (% CV)	19.1	13.5	11.2	14.1	9.9

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calculated as

$$\% \text{ Accuracy} = \left\{1 - \frac{(\text{nominal concentration} - \text{measured concentration})}{\text{nominal concentration}}\right\} \cdot 100$$

The acceptance criteria for accuracy is ±15% of the nominal concentration, but at the LLOQ an error of ±20% is permissible.

Precision

Precision is the determination of how close the repeated measurements of the same concentration are to one another. A minimum of three analyte concentrations that span the standard curve should be assessed, and at least five determinations or replicates should be conducted for each concentration. Precision is calculated as the coefficient of variation (% CV) following repeated measurements.

Precision (% CV)=(standard deviation/mean) • 100 (see Table 1)

The acceptance criteria for precision is a coefficient of variation of ±15%, but at the LLOQ a precision of ±20% is permissible. For the determination of both accuracy and precision, within-day (within-run) and between-day (between-run) determinations are made.

Recovery

Recovery is a measure of the ability of the extraction procedure to recover the drug spiked into the biological matrix. Recovery is determined by comparing the response of the analytical system to the analyte sample that was extracted according to the analytical method, with the detector response obtained from the same amount of pure authentic standard. The recovery of the analyte does not need to be 100%, nor is it a required element of method validation because, problems with recovery will be detected by unacceptable measures of accuracy and/or precision. However, during method development it is advisable to determine recovery in order to diagnose problems with the analytical assay which may occur. Furthermore, it is also advisable to determine the recovery of the internal standard independently, if one is used.

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Assessing the Effects of Sample Handling on Analyte Stability and Quantification

The determination of sample stability indicates the extent of drug or metabolite degradation that could be expected to occur as a result of sample handling. In extreme cases of degradation, this information could prompt the development of new sample-handling procedures. This is an important, although frequently under-appreciated characteristic of an analytical method. Typically, blood samples are collected according to a scheme similar to the following

1. Blood sample withdrawal at the study site; blood samples may be stored in ice for short periods.
2. Isolation of plasma from blood sample by centrifugation; this operation may take 10 to 20 minutes, and the centrifugation may or may not be refrigerated.
3. Plasma samples are then frozen and stored for some period.
4. Frozen plasma samples are transported to the analytical site; commercial carriers are usually employed for transportion and the samples are usually shipped on dry ice. The temperature at which the samples are shipped may differ from the storage temperature at the study site.
5. Plasma samples may be frozen at the analytical site for some period before analysis; storage temperatures at the analytical site may be different than those used at the study site or during transportation.
6. The plasma samples are thawed, and aliquots of the sample are processed and analyzed. The remaining plasma samples are refrozen. These remaining samples may be rethawed and reanalyzed at a later date.

This example illustrates that there are numerous opportunities for sample degradation that could ultimately lead to erroneous pharmacokinetic interpretations. Therefore, the chemical characteristics of the analyte should be considered during the development of standard operating procedures (SOPs) for sample collection. For example, the collection of samples for a pharmacokinetic study of nitroglycerin is quite challenging. The elimination half-life $(t_{1/2})$ of nitroglycerin is two minutes in vivo, and once a sample is withdrawn, the $t_{1/2}$ in blood is six minutes. Therefore, it is imperative that the plasma from these samples are isolated rapidly, under refrigerated conditions, and frozen immediately.

Another consideration that should be borne in mind is that stability testing should mimic the conditions of sample handling and storage to be used in the study. There have been examples in which long-term stability

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studies were conducted on samples stored at -70 °C. However, according to the SOPs established in the study protocol, the samples were stored at -20 °C in practice, and the results of the stability study were of limited value because the extent of sample degradation under the actual conditions of use were not assessed. The assessment of analyte stability should be addressed in the following discrete steps.

Freeze-thaw Stability

During the average study, it is likely that the samples may experience several freeze-thaw cycles, and it is important to know the sensitivity of the analyte to degradation resulting from this type of handling. This effect is assessed by assaying spiked samples after three freeze-thaw cycles. Study samples should be frozen for a minimum of 24 hours (at the temperatures planned for storage in the clinical study), then thawed at room temperature. Once completely thawed, the samples should be refrozen for a period of 12–24 hours. This cycle should then be repeated twice or more, and then the samples should be analyzed. Low and high concentrations of the drug should be assessed in triplicate.

Short-Term Room Temperature Stability

This characterization is meant to assess any degradation that may occur as the samples are maintained on the benchtop prior to and during sample processing (i.e., extraction, etc). Low and high concentrations of drug in triplicate should be maintained at room temperature for the period of time required for sample preparation and then analyzed.

Long-Term Stability

In this case, stability of the samples should be assessed according to the planned storage conditions (e.g., -70°C), but for periods that exceed the planned duration of storage. Three aliquots of low and high concentrations need to be assessed three times during the planned period of storage, and compared to the mean back-calculated concentrations of the sample determined on the first day of the study. Care should be taken to make samples with the necessary volume for repeated analyses. Interestingly, the Code of Federal Regulations stipulates that sufficient quantities of samples must be collected during a bioavailability (21 CFR 320.38) (11) or bioequivalence (21 CFR 320.63) [12] study and stored for *five years* from the date of NDA or ANDA submission. This regulation implies that longterm stability testing of the analyte should span this period as well. However, this may be practically impossible to achieve, and FDA does not require this step.

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Stock Solution Stability

The stability of the stock solution of the analyte which would be used to construct standard curves and quality control samples should be assessed following approximately six hours at room temperature, and following periods of refrigerated storage that are anticipated to be used during the study.

Post-Preparative Stability

This characteristic may also be referred to as autosampler stability. The stability of the processed samples should be assessed over the course of analysis (i.e., the run time) according to the conditions of use (e.g., room temperature or refrigerated autosampler). The stability assessments described above should also be performed for any internal standard or drug metabolites that may be measured in the assay, as well as the analyte of interest.

Monitoring Assay Quality During Practical Use

Once the method has been established and validated, it is ready for analytical use. However, as most current analytical methodologies employ automation to increase productivity, analytical runs have become very long (up to days). Therefore it is necessary ensure that the assay continues to perform according to the specifications determined during the validation stage throughout each analytical run. This is accomplished by making and including quality control samples or calibrators (QC) of known concentrations that can be interspersed with the calibration standards and the clinical samples in each analytical run. The QC samples allow the analyst to monitor the accuracy and precision of the method while it is in use. QC samples are standards that are made of known quantities of drug that is spiked into naïve matrix. A minimum of three concentrations that bracket the standard curve should be prepared. The first QC sample should be within 3×of LLOQ, the second QC sample should be mid-range and the third QC sample at the upper end of the standard curve should be included. The QC samples should be run in replicate. The QC samples should be interspersed with the clinical samples and the standard curve calibrators, but there is no consensus on how frequently QC samples should be incorporated. The FDA recommends that 5% of the samples in the run should be QC samples, but six QC samples are the absolute minimum for any run. Both standard calibrators and QC samples should be arranged to detect assay drift. In order to accept the analytical run, two-thirds of the QC samples must be within 15% of their nominal values. For example, if six QC

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samples were analyzed (two low QCs, two mid-QCs, and two high QCs) and one replicate each at the mid- and high QC concentrations were greater or less than 15% of nominal, the run would be deemed acceptable. However, if two replicates at the same QC concentration failed (e.g., both mid-QC samples in the example above), or more than two QC samples failed, the analytical run would be rejected.

In addition to monitoring the method performance, it is also good practice to include QC samples with the samples during storage. QC samples can be prepared at the same time the samples are processed, and stored with the samples to monitor storage conditions. This practice is useful to guard against unforeseeable events, such as a power outage that affects freezer function. This use of QC samples, although advisable, is not a requirement of analytical method validation.

ANALYTICAL METHOD VALIDATION DATA FOR SUBMISSION TO FDA

Information that should be submitted in an NDA or an ANDA for the analytical method validation should include the following:

- Summaries: A summary table that lists the validation studies by title and number, and a table of the assay methods used in the study (s).
- Method Establishment information: This should include a description of the analytical method(s), evidence of analyte purity, description of stability studies, description and tabulation of accuracy and precision determinations, cross-validation studies if necessary, legible chromatograms, or mass spectrograms including blanks (up to 20% of chromatograms from three serial patients for pivotal bioequivalence studies), and a list of deviations from protocols and explanations for these deviations.
- Application of the validated method: Summary table of sample handling, summary table of clinical or preclinical samples, equations used, table of calibration curve data, summary tables of intra and inter assay accuracy and precision, and of QC samples, reasons for missing samples, reanalyzed samples, and reintegrated samples.

PARTIAL VALIDATIONS AND CROSS-VALIDATIONS

The steps described above detail the process of complete or full validation that is necessary for the development of a new analytical method. However,

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there are two other method validation situations that require some discussion. These situations are partial validations and cross validations.

Partial Validations

Periodically changes to a validated assay are necessitated for a variety of reasons. For instance, due to protein binding, it may be necessary to switch from heparin as an anticoagulant to EDTA. This apparently small change to the validated assay may alter its performance and it is necessary to demonstrate whether or not the characteristics of the assay have changed. A full validation is likely not necessary, as a partial validation will suffice to address the question. Unfortunately, the extent of partial validation is left to the discretion of the analyst. Partial validations may range from one intraassay accuracy and precision determination, to almost a complete validation. A reasonable suggestion is that partial validations should basically consist of selectivity, accuracy, and precision determinations. Once this step is completed, the analyst may decide on the need for further validation of the modified assay. Some of the situations where partial validations should be considered are listed in the FDA Guidance. This list is not exhaustive, but it describes the most likely partial validation situations. Some of these scenarios are:

- Method transfer between labs or analysts
- Change in detection system
- Change in anticoagulants
- Change within matrix within species (e.g., human plasma to human urine)
- Change of species within matrix (e.g., rat plasma to mouse plasma)
- Changes in sample processing
- Change in concentration range
- Instrument or platform changes
- Limited sample volumes
- Rare matrices
- Selectivity demonstration of analyte in presence of concomitant medications or in the presence of metabolites

Cross Validation

Cross validation of analytical methods is a special case. Cross validations are a comparison of the validation parameters of two or more bioanalytical methods. Generally, most bioanalysts develop and validate an analytical method prior to the start of a clinical study. However, there are two

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situations that can arise where cross validations should be conducted: when two or more analytical methods are used to generate data within a single study (including situations where one method was significantly changed during the study), or when two or more analytical laboratories are used to generate data within a single study. In addition, the analyst should consider cross validation in cases where significantly different analytical methods were used to generate data in different studies, if both studies produced data of pivotal importance to the NDA. Unfortunately, there is no uniformly accepted format for conducting cross validations. However, there are two general approaches, which are quite similar. First, spiked samples of low, medium, and high concentrations are simply analyzed by both methods and compared.

Alternatively, clinical samples are analyzed by the different methodologies and plotted against each other (see Fig. 7). Both methods should provide the same value, and the slope of the line should equal unity. This approach also allows certain statistical comparisons to be made [13]. Generally, the FDA recommends that both spiked samples and patients samples should be compared between methods. However, it is also unlikely that both methods will be exactly equal. The question then is how much difference is acceptable. This issue has not been fully addressed, but usually the ± 15/20% criteria used for accuracy and precision has been applied. It is

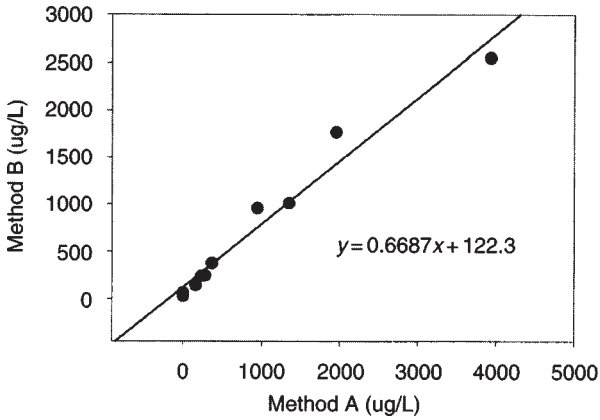

FIGURE 7 Cross validation. A set of patient samples were analyzed with two different methods, A and B. The concentrations determined by each method are plotted against one another. Ideally, if both methods were equal, they would produce the same concentrations and a slope equal to one. In this case the slope is 0.66, which indicates that Method A reports higher concentrations than Method B.

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advisable that the bioanalyst assess the objectives of the clinical study, and set the requirements for cross validation appropriately.

CONCLUSIONS

The guidelines set forth in the FDA Guidance provide the framework that can be applied to most cases of analytical method validation, regardless of the analytical principle employed, and is most likely to assure the necessary reliability of an analytical method. However, it is understood that there are situations and methodologies where a validation cannot produce the degree of accuracy or precision described. The over-riding question that needs to be addressed by the bioanalyst is whether the analytical method reliably meets the need(s) of the clinical study. In these cases, if the bioanalyst has demonstrated due diligence and effort in method development, and the reliability of assay given the requirements of the study, validations with lower standards may also be deemed acceptable.

REGULATORY WEBPAGES

Australia, Therapeutic Goods Administration: www.health.gov.au/tga/
Canada, Therapeutic Products Directorate: www.hc-sc.gc.ca/hpfb-dgpsa/
Europe,EMEA:eudraportal.eudra.org/
International Committee on Harmonization: www.ifpma.org/ichl.html
Japan, Ministry of Health and Welfare: www.mhw.go.jp/english/index.html
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9

Studies of the Basic Pharmacokinetic Properties of a Drug—a Regulatory Perspective

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INTRODUCTION

This chapter concerns basic pharmacokinetic studies that are essential for understanding the characteristics of a new chemical entity; however, all types of studies are not covered by specific regulatory guidance documents or regulations. The majority of these studies are performed early in the clinical development of a new chemical entity. Single-dose studies form the basis of the pharmacokinetic knowledge needed for a rational drug development program. Repeated-dose studies confirm results obtained after single-dose administration, but can also reveal time-dependencies, nonlinearity, and self-induction/inhibition in the pharmacokinetics of a drug. If adequate information is captured early in development, the need for

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additional Phase I studies, e.g., to elucidate apparent inconsistencies in basic pharmacokinetic properties observed in early studies, may be reduced, and the appropriate designs of early Phase II studies can be selected with added confidence. If essential pharmacokinetic knowledge is obtained early on in the development, a potentially negative result of an early proof-of-concept study in the target (patient) population would more likely reflect drug effects rather than a miscalculation of the dosage regimen. It is desirable that the drug levels should be monitored in such a proof-of-concept study, to get insight and knowledge of preliminary exposure (pharmacokinetic)-response (pharmacodynamic) relationships of the drug. It is also advisable to investigate potential exposure-response relationships throughout all stages of drug development. Readers are referred to Chapters 10 and 11 for a more detailed description of such studies.

The studies that will be discussed in this chapter are early safety and tolerability studies, mass balance or ADME studies, dose proportionality studies, bioavailability studies, food interaction studies, and repeated dose studies. In the review of a new drug application (NDA), evaluation of the validation of the bioanalytical methods such as specificity, sensitivity, limits of detection, and quantitation plays an important role in the overall assessment of the validity of the pharmacokinetic data. Chapter 8 describes the analytical method validations that should be performed prior to conducting these studies.

The Guidance documents issued by the U.S. Food and Drug Administration (FDA) referred to in this chapter can be found on the FDA's website www.fda.gov/cder. A summary of the Code of Federal Regulations (CFRs) quoted in this chapter can be found in Chapter 3, or in the Federal Register. For specific regulations by other regulatory agencies in the world, readers are referred to the specific agency's website and encouraged to contact the appropriate agency for additional information they may need.

SINGLE-DOSE STUDIES

The major part of the basic properties of a chemical entity can be extrapolated from single-dose studies if the pharmacokinetics of the drug are linear. Linear pharmacokinetics is described by an increase in dose that is followed by a proportional increase in exposure of the drug (e.g., the area under the plasma concentration-time curve), over the anticipated therapeutic dose interval. The basic pharmacokinetic parameters of a drug from the single-dose studies can then be used for predictions of drug exposure after repeated doses, after various dosing regimens [1].

Indications of nonlinear pharmacokinetics should be investigated early to determine if the cause is related to absorption, distribution,

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metabolism, or excretion processes. It is generally recommended that the pharmacokinetic studies are performed in fasting subjects (overnight fast), to reduce the influence of potentially confounding factors elicited by concomitant food intake. On the other hand, it is most desirable that potential influence of food on the pharmacokinetics of the drug also is investigated early on in the drug development program. This information facilitates appropriate recommendations as how the drug should be administered in the Phase II or Phase III trials in the target patient populations.

Safety and Tolerability

The initial study, where first dose is administered in humans, yields valuable information regarding basic pharmacokinetic properties of a new chemical entity, and can give indications about potential nonlinearities in the pharmacokinetics. This safety and tolerability study is usually conducted in healthy adult volunteers, where subjects are administered escalating doses of the drug, starting from low doses that are increased in a stepwise manner. Generally, safety parameters are intensively monitored, and volunteers scheduled for the next dose level are not dosed until a safety evaluation from the previous cohort of subjects is completed. The maximum dose in the study is usually not predetermined, but is limited by adverse events or by predetermined stopping rules. Recommendations of the preclinical toxicological studies that should be completed and evaluated before the first human trial is initiated are described in the ICH Guidance "Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals" [2].

Choice of Dose

The starting dose and the subsequent dose increments are generally chosen according to the preclinical pharmacological and toxicological results. The less toxic effects a drug has shown to produce, the larger dose increments can be made, at least during the initial part of the dose-escalating trial. Criteria for stopping rules of the dose-escalation, i.e., the maximal dose given in the study, should be predetermined and specified in the protocol, as far as possible. The stopping rules may include a number of subjects that experience moderate to severe adverse events, plasma levels where preclinical toxicological findings limit further dose increases, or established surrogate maximal endpoints that have been reached. The first safety and tolerability study can provide considerable insight regarding the therapeutic index of a drug if an adequate dose range is explored.

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Assessments of the exposure-response relationships for a new chemical entity in a preclinical animal model may give sound directions for the therapeutic concentration (exposure)-effect (response) relationships to be evaluated in the first safety and tolerability study, as well as subsequent studies. Although surrogate endpoints or biochemical markers usually are used as an alternative to the clinical endpoints used in the later confirmatory Phase III trials, early information regarding exposureresponse correlations from both preclinical animal and healthy volunteer studies could aid further drug development. Naturally, the chosen surrogate endpoints or markers should capture information that is considered to be applicable to the future patient therapy. The exposure-response relation-ships determined in the preclinical pharmacological and toxicological studies can also guide the magnitude of dose escalation steps in the first study. A steep exposure-response correlation calls for smaller dose increases compared to a more shallow correlation between dose or concentration and pharmacological or toxic effects of the drug. However, the assumption is that the metabolism and activity of the drug and metabolites are similar in the animal species and humans. For example, a particular metabolite contributing towards toxicological or pharmacological effects may be formed in humans but not in animals, which may, in part, invalidate predictions based on preclinical observations.

Interspecies scaling is used as an instrument to predict pharmacokinetic parameters and exposure in humans. Two techniques, physiologic and allometric scaling, and more recently, allometric scaling in combination with in vitro-in vivo correlations, are extensively described in the literature [3–6]. Interspecies scaling techniques are also described in detail in Chapter 7. The allometric scaling approach may be very useful as an aid for predictions of the dose interval to be investigated in the first safety and tolerability study. At present, there are no requirements or final guidance documents regarding the use of scaling techniques for dose selection in Phase I studies. However, the FDA has recently published a draft Guidance [7], which mainly focuses on an algorithm for calculations of the maximum recommended starting dose (MRSD) in humans from animal data. The described algorithm for these estimations include appropriate safety margins for the MRSD is based on available no observed effect levels (NOEL) in animals. Allometric scaling and modeling are also considered, and it is recommended that an adequate safety factor for the MRSD is also included, if such approaches are chosen.

A combination of allometric scaling techniques and knowledge of the exposure-response relationships has indeed proved to be worthwhile. In a survey from one major pharmaceutical company it was estimated that timesavings of two weeks to six months could be accomplished in the first safety and tolerability study by utilizing exposure-response correlations and

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allometric scaling techniques from preclinical studies [8]. The major advantage was a reduction of dose steps in the low, subtherapeutic dose range.

Study Population

The study population in the first safety and tolerability study is usually healthy, adult male and female volunteers aged 18–45 years old, with normal weight in proportion to their height. Since the preclinical reproduction toxicity studies may not have been completed when the first human safety study is performed, women of childbearing potential may be excluded from that study population. However, it is highly recommended that women are included as early as possible in the first human clinical pharmacology studies [9–11].

As a matter of fact, as stated in the ICH Guidance document ICH M3 [2], there are regional differences across the world in the recommended timing of reproduction toxicity studies to support the inclusion of women of childbearing potential into human trials. The regional differences outlined in ICH M3 are as follows:

- *The United States:* Women of childbearing potential may be included into carefully monitored trials before the reproduction toxicity studies have been completed. Recommended safety measures include pregnancy testing, the uses of a method of birth control considered as highly effective, and study entry after a verified menstrual period.
- *The European Union:* The evaluation of embryo-fetal development should be completed prior to Phase I trials, and female fertility before Phase III trials are initiated, in women of childbearing potential.
- *Japan:* Assessment of female fertility and embryo-fetal development should be completed before women using birth control are included in any type of trial. Permanently sterilized or postmenopausal women may be included into trials before reproduction toxicity studies have been completed, if the appropriate repeated toxicity studies have been performed, where any toxicity related to the female reproductive organs have been evaluated. A male fertility trial should be completed before the Phase III trials are started.

If the target patient population only encompasses a certain specific population, e.g., women for oral contraceptives or hormone-replacement therapy, or drugs for Alzheimer's disease in the elderly, more adequate

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information could be gathered by performing the early Phase I studies in the intended target population (e.g., women or elderly subjects). In certain cases when the toxicity of the drug is expected to be high, e.g., drugs intended for treatment of cancer, it might be unethical to perform any trials in healthy volunteers, thereby exposing healthy subjects to drugs that may cause undue harm. All these factors should be considered at the time of design of these studies.

Study Design

The first safety and tolerability study in humans is usually performed in single escalating dose, open, or single-blind, parallel design. The number of subjects included in each dose level is generally limited (n=3–8), where the number of subjects is increased at higher dose levels. A parallel design is usually chosen to increase the number of subjects that are exposed to the drug, thereby maximize early safety information regarding the pharmacological or toxicological effects on variables such as vital signs, clinical chemistry, and adverse events. A parallel group design may also reduce the risk for the individual volunteer if unexpected adverse events occur where repeated exposures may augment the unforeseen adverse events. A limited placebo control group can also be valuable, especially if the pharmaceutical formulation contains an excipient or a vehicle that may elicit a pharmacological or a toxicological response.

An adequate number of blood samples is recommended to ensure, as far as possible, that a full plasma concentration-time profile is attained.

Data Analysis

Accurate information regarding the maximum drug plasma concentration (C_{max}), area under the plasma concentration-time curve (AUC), terminal half-life ($t_{½}$) of the drug, and the interindividual variability are valuable for future study designs. The methods for calculation of the parameters are discussed in the section "Data Analysis" on page 199 of this chapter. Although the number of subjects usually is limited in the first human study, initial information regarding dose linearity, i.e., proportional increases in exposure (C_{max} and/or AUC) with increasing doses, can be made. An attempt to evaluate information regarding relationships between plasma concentrations of drug and pharmacological effects, surrogate markers, or adverse events is also valuable. Any information regarding such relationships would enhance appropriate future study designs.

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ADME (mass balance)

The absorption-distribution-metabolism-elimination (ADME) study in humans is not only one of the most informative, but also one of the most labor intensive, Phase I studies. Although in vitro studies yield qualitative information regarding metabolism across species, quantitative information can only be obtained from in vivo studies. The timing of the ADME study in relation to other studies in the clinical development program varies. However, the earlier the study is performed, the more useful are the results from the study. Early information regarding major metabolites and excretion patterns is essential for rational planning of studies, e.g., for special populations. Since elucidation of metabolic patterns may be timeconsuming, it is advantageous to initiate the ADME study as one of the first Phase I studies. It is obvious however, that the choice of dose and sampling collection at appropriate time intervals is essential for a good outcome of the study, therefore knowledge about the basic pharmacokinetic properties of the drug should be attained before the ADME study is initiated.

Choice of Dose

The dose of the radiolabeled drug should be kept as low as possible. Information regarding tissue distribution in animals, e.g., from whole body autoradiography studies, provides valuable information about high drug accumulation in specific tissues, as well as the time course of elimination from specific tissues. The information can also be utilized in the risk assessment of the use of radioactive isotopes for human studies. The regulations regarding the use of isotopes in human research vary between different countries. Dosimetry calculations to estimate exposure in different tissues need to be performed, and in general the protocol has to be approved by a Radioactive Drug Research Committee as well as an Investigational Research Committee. In the United States, the rules for the use of radiolabeled drugs in research can be found in 21 CFR 361.1, and the reader is also referred to a related overview by Dain et al. [12].

The choice of radiolabel for the drug is usually dependent on the isotope that was chosen for the mass balance studies in the animal species. The same isotope should be used in the human in vivo study to enable crossspecies comparisons of metabolic patterns. This is important, since the metabolic pattern should be similar between the animal species chosen for the preclinical carcinogenicity and long-term toxicity studies and humans. If the metabolic profiles differ substantially between humans and animals, additional (preclinical) studies may be needed. For example, if a major metabolite is formed in humans, which has not been observed in animal

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studies, then this metabolite may have to be synthesized and administered to animals to assess the pharmacological and toxicological properties of the particular metabolite. In such cases, the appropriate regulatory agency should be contacted to get their guidance on which additional studies may be needed, or to discuss the adequacy of additional study protocol proposals. The radiolabel should be properly positioned in the molecule to yield relevant information regarding the drug metabolism. The radiochemical purity is also important, especially for protein-binding assessments of highly (>99%) protein-bound drugs [13].

Study Population

The ADME study is usually performed in healthy, adult, male volunteers, 18–45 years of age. Women are traditionally excluded due to the potential risks associated by exposing females of childbearing potential to a yet unapproved, radiolabeled drug. By the same token, certain investigators limit the lower age limits of the male volunteers to an age arbitrarily chosen above 18, for example an age of 35 years, and may extend the upper age limit to 60 years. The number of subjects is usually low (n=4–8), but some caution should be used in keeping the number of subjects high enough, so that the results will be informative. If the drug has shown highly variable pharmacokinetics in earlier studies, a larger number of subjects may have to be included in the study.

Study Design

The optimal design of an ADME study is a crossover, or a parallel group, study where an intravenous (IV) dose serves as a reference to the enteral (e.g., oral, rectal, or sublingual) or other parenteral (e.g., topical or pulmonary) routes of administration. Even if the development of the new chemical entity is only focused on, e.g., an oral route of administration, the pharmacokinetic information from an IV dose will significantly enhance the understanding of the pharmacokinetics of the drug, especially information regarding absorption processes, presystemic metabolism, and first-pass effects. However, a study design, where only one route of administration is chosen, would be satisfactory, although more limited information regarding the ADME processes will be collected.

Blood and plasma samples, aliquots of urine and feces, and in certain cases expiration air, are collected over an extended period of time. The time period for collection of biological specimens is obviously governed by the terminal half-life of the drug and/or metabolite(s), and can be determined by "on the spot" quick-counts of radioactivity in, e.g., urine or feces. The blood-sampling period is usually terminated well ahead of urine and feces

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collection, where the latter usually continues for 7–10 terminal half-lives of the drug or metabolite(s). It is essential that the recovery of the total radioactivity in the different biological fluids is 85–90% or above, therefore strict provisions regarding sampling collection need to be made. The volunteers need to be fully informed and understand the importance of complete collection of urine and feces specimens, and comply with the instructions.

The metabolite identification is performed in the biological samples after extraction and separation (e.g., by fractional collection). Metabolite identification should be attempted in all the collected biological specimens (e.g., blood or plasma, urine, feces). The metabolite structures are generally identified by use of liquid chromatography-(tandem) mass spectrometry methods [14]. Accelerator mass spectrometry (ACL), which has been used for areas such as age determination of archeological objects, has recently been applied in biomedical research, e.g., ADME studies [15, 16]. The main advantage with this technique is a very high sensitivity and precision, which permits the use of extremely low doses of radiolabeled materials and quantitation of low levels of radioactivity. However, this promising technique is not yet used routinely, and may require further validation. All analytical methods need to be adequately assessed, as described in Chapter 8.

Data Analysis

The data analysis is usually extensive. Graphs of the time-course of excretion (e.g., urine and feces) and plasma/blood profiles of total radioactivity, as well as of each analyte should be constructed. The ratio of parent compound and each metabolite to total radioactivity may also be calculated.

Pharmacokinetic parameters, e.g., AUC, C_{max}, t_{max}, total clearance (CL), renal CL, terminal half-life, apparent volume(s) of distribution *(V_γ)*, and amount of drug excreted unchanged in urine *(A_e)*, should be calculated for the drug. The corresponding parameters should, if possible, be calculated for the major metabolite(s). If an IV dose is administered, absolute bioavailability and actual CL and V_γ values can be calculated. An IV dose can be extremely valuable, since any quantitative differences in metabolism, excretion patterns and CL between IV and oral administration, as well as a measure of the absolute bioavailability and extraction ratio, will aid the understanding of the disposition of the drug. Incomplete absorption can be detected from differences in excretion patterns and presystemic metabolism can be detected from different metabolite/parent ratios between different routes of administration. The report is enhanced when it contains clear graphs and tables of both individual and average data, as well as summary statistics. Due to the exploratory nature of the ADME study only descriptive

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statistics are expected. If the information is available, a scheme of the proposed metabolic pathways in humans adds valuable information to the study report.

Bioavailability

Definitions

Absorption of the active moiety is a stipulation for systemically acting drugs that are administered by an extravascular route [1]. Bioavailability is defined as the rate and extent of absorption of the intact drug or active moiety. Studies that concern the evaluation of dose-linearity, potential food-drug interactions, and the pharmacokinetics after repeated administration are discussed in subsequent sections of this chapter. Alternative approaches, i.e., pharmacodynamic studies, to those described in this chapter might be necessary for locally acting drugs, where systemic exposure is not intended and cannot be assessed. However, if the bioavailability (or bioequivalence) of a drug can be determined by a pharmacokinetic study, a pharmacodynamic approach is not recommended.

Bioavailability and especially bioequivalence studies are generally performed throughout a product's life cycle, both before and after the drug approval. Bioequivalence studies are the principal basis for approval of abbreviated NDAs for generic drugs. These studies are essential for both efficacy and safety, by demonstrating that the pharmaceutical formulation gives reproducible drug exposure, and intended plasma levels of the active moiety. Bioequivalence studies are discussed in detail in the Biopharmaceutics section, and will not be discussed in this chapter.

The European Agency for the Evaluation of Medicinal Products (EMEA) has issued a new guidance document regarding investigations of bioavailability and bioequivalence in July 2001 [17]. In the United States, the requirements for bioavailability and bioequivalence studies for product approval are described by the Code of Federal Regulations (21 CFR 320), and more details are found in Chapter 2. In 21 CFR 320.1, bioavailability is defined as "the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended -to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action."

As an additional support for adequate designs of bioavailability (and bioequivalence) studies, FDA has published several guidance documents regarding the general principles for these studies:

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- "Bioavailability and Bioequivalence Studies for Orally Administered Drug Products—General Considerations" (Revision 1, March 2003)
- "Food-Effect Bioavailability and Fed Bioequivalence Studies" (December 2002)
- "Statistical Approaches to Establishing Bioequivalence" (January 2001)
- "Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations" (September 1997)
- "Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System" (August 2000)

The guidance documents relating to bioequivalence and conditions where waivers are granted in lieu of in vivo studies are discussed in detail in the chapters in the Biopharmaceutics section of this book. It should be noted that the guidance documents are recommendations, and reflects the current thinking of the FDA. Alternative approaches than those recommended in the guidance documents may be employed if the requirements of the statutes in 21 CFR 320 are fulfilled.

Methods

The most commonly used method to determine the rate of absorption is by reporting the time *(t_{max})* to reach the (observed) peak plasma concentration *(C_{max})* of drug after dose intake. The observed C_{max} of the administered drug characterizes the peak exposure after dose intake. Other methods to determine the rate of absorption may be employed, which may be more meaningful for the comprehension of the absorption processes of the drug, since t_{max} and C_{max} are governed by both absorption and elimination processes. Examples of other methods are deconvolution or calculations of the absorption rate constant (ka), and can also be utilized [18].

The extent or completeness of absorption of intact drug or the active moiety is usually expressed by the area under the plasma concentration-time curve, AUC, as a quantitation of exposure. Comparative bioavailability is expressed as a fraction (or percent) of the administered dose, where another pharmaceutical formulation or route of administration serves as reference. Comparative bioavailability (F) is calculated as:

$$F(\%) = \frac{\text{Dose}_{\text{reference}}}{\text{Dose}_{\text{test}}} \cdot \frac{\text{AUC}_{\text{test}}}{\text{AUC}_{\text{reference}}} \cdot 100,$$

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where AUC denotes the area under the plasma concentration-time curve, and dose adjustments are performed if unequal doses of the test and reference drugs are administered. Alternative biological fluids, e.g., whole blood or urine, can also be used for the determination of bioavailability. Absolute bioavailability (F) is determined after administration of an intravenous reference dose, where the intravenously administered dose is assumed to be 100% bioavailable. Relative bioavailability *(F_{rel})* is determined when the reference dose is administered extravascularly, e.g., as an oral solution or a suspension. Early indications of a lower F_{rel} than expected may call for additional modifications of the drug substance where ultra micronization or other measures may increase the in vivo absorption of the drug.

In certain cases, absorption is the slowest, rate-limiting step in the disposition of a drug. Differences in terminal $t^1/_2$ of the drug after different routes of administration may indicate rate-limiting absorption processes [1]. Again, an intravenous reference dose is one of the most straightforward ways to determine the basic pharmacokinetic properties of the drug or formulation, since the intravenous route of administration circumvents all absorption processes.

Relative or absolute bioavailability of the dosage form should to be established. In early stages of drug development, the oral tablet formulations are usually of immediate release (IR) character, and an oral solution, or suspension, are used as the reference if an intravenous formulation is not available. This study can be valuable as a point of reference, if subsequent modifications and optimizations are made to the dosage form during further drug development. It is possible to link formulation changes by bioavailability studies between formulations, and in vitro dissolution comparisons may also preclude in vivo studies if only minor modifications are made. However, major changes between clinical trial formulations and/or the formulation intended for commercial use may warrant bioequivalence studies (see related chapters in the Biopharmaceutics section).

Study Population

Bioavailability studies are usually performed in healthy, adult volunteers, above 18 years of age. Inclusion of equal numbers of men and women, or volunteers resembling the patient target population (e.g., elderly), is encouraged. The number of subjects participating in the study should be based on earlier studies where intersubject, and, if available, intrasubject variabilities have been determined.

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Study Design

A single-dose, randomized, crossover design is the most common choice for a bioavailability study. Study drug should be administered with 240 mL (8 oz) of water after overnight fast and standardized meals should not be served until four hours post-dose. Water ad lib is allowed ± 1 hour of dose intake.

In rare cases, a parallel-group design may be selected instead of a crossover design. Drugs with a long terminal half-life may preclude the choice of a crossover design, due to practical aspects of sample collection. For a comparative bioavailability study of a drug with a long terminal halflife, an alternative design, e.g., the "semi-simultaneous" method, may be considered. In the "semi-simultaneous" approach, the test and reference doses are administered at one occasion, but the doses are separated by a certain time interval and no washout period is employed [19]. However, it is recommended that any nontraditional study design should be discussed with the regulatory agencies prior to study initiation, to determine the regulatory view on the appropriateness of the specific design.

Blood samples should be collected to adequately describe the full plasma/serum drug concentration profile, including absorption, distribution, and elimination. It is essential to characterize the absorption phase (predose and 1–3 samples before C_{max}), as well as the terminal phase (≥3 samples) of the plasma concentration-time profile, where sampling should be continued up to at least three terminal $t_{½}$ of the drug/active moieties. Investigational periods should be separated by an adequate washout interval ($>5t_{½}$) to ensure that elimination is complete before the second dose is administered.

Data Analysis

Standard pharmacokinetic parameters, area under the plasma concentration-time curve (AUQ_t and AUC_∞), observed maximum plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}), elimination rate constant (γ_z), and terminal $t_{½}$ are routinely calculated for the intact drug as well as any active metabolites. AUQ_t is calculated from time zero (time of dose intake) to time t, where t is the last time-point with a measurable drug concentration (C_t) in plasma. AUQ_t is calculated by the linear or log-linear trapezoidal method. AUC_∞ is calculated from time zero to infinity, where $AUC_\infty = AUQ_t + C_t/\gamma_Z$.

As stated earlier, other methods to determine the rate of absorption better than t_{max} and C_{max} may be employed. For regulatory purposes, however, the observed C_{max} and t_{max} should always be included in the data analysis and

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report. Compartmental methods may also be used for calculations of AUC, but in general, noncompartmental methods, such as the trapezoidal method, are preferred. As a matter of fact, the European Guidance [17] does not recommend the sole use of compartmental calculation methods for the analysis of bioavailability or bioequivalence studies.

For a comparative bioavailability study, 90% confidence intervals should be constructed for the log-transformed ratios of AUC_t, AUC_∞, and C_{max} for the test and reference formulations. If unequal doses of test and reference formulations are administered, dose corrections should be included in the calculations. Although the objective of a comparative bioavailability study differs from confirmatory bioequivalence studies, i.e., 80–125% as a pass criterion does not have to be fulfilled, it is highly recommended that 90% confidence intervals for ratios of AUC_t, AUC_∞, and C_{max} for the test and reference formulations be reported. The report should contain clear graphs and tables of both individual and average data, as well as summary statistics.

Food-Drug Interactions

Concomitant food and drug intake has the potential to cause altered drug absorption due to physicochemical and/or physiological reasons [20]. The absorption process is in part dependent on the physicochemical properties of a drug, such as pKa, rate of dissolution, and chemical stability, which all may be altered by concomitant food intake. Certain effects may readily be predicted from the chemical properties of a molecule, e.g., an acid-labile structure will be subject to an increased rate of degradation due to prolonged residence time in the stomach, where absorption of the drug will be decreased after concomitant food intake. A suitable pharmaceutical formulation can prevent such a phenomenon by, for example, enteric coating of the oral tablet to protect the drug substance to premature degradation.

Food also alters gastrointestinal physiology compared to the fasting state, by delaying gastric emptying, changing pH in parts of the gastrointestinal tract and increasing visceral blood flow, among other effects. All these changes may modify the absorption of the drug, but some might also be quite easily predicted by examining the inherent chemical or pharmacokinetic properties of the substance. The composition of the meal, such as the fat, protein, and overall caloric content can also influence the magnitude of an observed interaction. The FDA has recently published a guidance document entitled "Food-Effect Bioavailability and Fed Bioequivalence Studies" [20], which is available on the FDA's website: www.fda.gov/cder.

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Choice of Dose and Composition of the Meal

A study investigating the potential influence of concomitant food intake should be performed under conditions that really stresses the system, that is a "worst case" approach should be used. Therefore, the highest dose in the expected therapeutic range should be chosen. A sound justification for the use of a lower dose strength is recommended, e.g., tolerability problems that precludes dosing at the highest dose level without previous dose titration starting at a lower level. If a modified release (MR) formulation has been developed, in vitro dissolution testing can be substituted for an in vivo study for other, usually the lower, strengths of the MR tablets. If the in vitro release profiles between the MR formulations differ, or the excipients differ qualitatively between the dosage strengths, additional in vivo food studies may be required for the other dosage strengths.

The composition of the meal should be of high caloric content (approximately 800–1000 calories) where 50% of the content consists of fat. The FDA gives an example of test meal, which fulfills these criteria, which is composed of two eggs fried in butter, two strips of bacon, two buttered slices of toast, four ounces (about 110g) of hash brown potatoes, and eight ounces (240 mL) of whole milk [20]. This meal gives about 150 calories from protein, 250 calories from carbohydrates, and 500–600 calories from fat. Alternate meal compositions can be used, but it is important that the proportions of fat, protein, and carbohydrates are kept to give a similar caloric content to the proposed test meal. The description of the meal should be included in both the protocol and the final report.

One may argue that the described breakfast is not an appropriate test meal for the vast majority of patients, since only a fraction of any population eats this type of breakfast. However, the purpose of the test meal is to study the effects of maximal perturbations created by concomitant food intake, both with respect to interaction between the drug, the pharmaceu-tical formulation, and the nutritional content of the meal. The high caloric content, in part originating from the high fat content, will also amplify the physiological effects of the test meal, e.g., the delay in gastric emptying and the increase in splanchnic blood flow.

Study Population

As described in the previous section (Bioavailability) the study is usually performed in healthy, adult male and female volunteers, above 18 years of age, unless the study is conducted in the target patient population. It is advisable to perform the, study in the target patient population if the indication of the orally administered drug is to treat a disease likely to alter drug absorption, e.g., inflammatory bowel disease. The sample size should

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be based on earlier determinations of intersubject variability, although it is recommended that a minimum of 12 subjects is included in the study.

Study Design

The most commonly used study design is a balanced, randomized, two-way crossover study, analogous to a bioavailability study, as described in Section 2.3.4 of this chapter. The subjects are given a single dose of the study drug in the fasting state (reference) and after a meal (test). Both the treatments should be preceded by an overnight fast (at least 10 hours), and the treatments should be separated by an adequate washout period.

- Reference treatment (fasting state): The drug should be administered with 240 mL (8 oz) of water. Water intake is permitted ad lib, except within ± 1 hour of drug intake, but standardized meals should not be served until four hours post-dose.
- Test treatment (fed state): The test meal should be consumed within a prespecified time interval (30 min) and the study drug should be administered with 240 mL (8 oz) of water immediately *after* completion of the meal. Water intake is permitted ad lib, except within ± 1 hour of drug intake, but standardized meals should not be served until four hours post-dose.

Additional studies might be necessary if an undesired food-drug interaction is observed which warrants special dosing recommendations regarding the timing of the meal in relation to dose intake. Especially, if the pharmacological effects are mainly related to peak concentrations rather than total exposure of the drug, and concomitant food intake reduces the C_{max} of the drug, the optimal time interval between the meal and dose intake should be explored to reduce the risk of therapeutic failure.

Data Analysis

Standard pharmacokinetic parameters, C_{max}, t_{max}, lag time (for delayed release products), AUQ_t, and AUC_∞, should be calculated for the intact drug and it is also valuable to calculate these parameters for major, active metabolite(s). The terminal half-life should also be reported. The reader is referred to the section "Data Analysis" on page 199 of this chapter, for a more detailed description of the calculations. The report should contain clear graphs and tables of both individual and average data, as well as summary statistics. The evaluation of the absence or presence of a food effect is based on the 90% confidence intervals (CI) for the ratio of the means of the test (fed) and reference (fasting) conditions of C_{max} and AUG.

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Absence of a food effect is concluded when the 90% CI for the ratio of the population geometric means (based on log-transformed data) met the limits of 80–125% for AUC and C_{max}.

If a food effect has been observed (>20% difference in AUC and C_{max} between fed and fasting states), the clinical relevance of this finding should be considered in relation to the dose (or exposure)-response relationships of the drug. The dosing recommendations should reflect the optimal timing of food intake in relation to drug administration, so the intended therapeutic effects of the drug are maintained. The clinical relevance of an observed change in the rate of absorption (t_{max} or lag time) between the fed and fasted states should also be considered and addressed in an NDA submission. Regulations regarding labeling requirements in the United States can be found in 21 CFR 201. The evidence of absence or documented food effects should be stated in the product labeling for the drug, and the "Dosage and Administration" section of the labeling should provide the instructions for drug administration in relation to food.

Timing of the Study

The objective of an investigation regarding the influence of food intake can be related to the drug substance in itself, or also be related to the pharmaceutical formulation. Early identification of a food effect is of value to optimize dosing recommendations in subsequent clinical trials or serve as a basis for attempts to minimize influence of the food by modification of the drug substance (e.g., micronization) or the pharmaceutical formulation. From a regulatory perspective, the information regarding food effects in a submission should be based on the to-be-marketed pharmaceutical formulation.

For an IR formulation, a study that indicates a substantial food effect performed early in development using a prototype IR formulation might not need to be repeated at a later stage. However, such a conclusion needs to be ascertained by reasonable information that shows that the food effect or absence thereof is due to the drug substance and not the formulation or processing factors. A food-effect study for a modified release (MR) formulation should always be performed on the highest dose strength of the to-be-marketed pharmaceutical formulation, unless tolerability or safety concerns preclude administration of the highest dose strength.

It should be noted that the conduct of the pivotal clinical (Phase III) studies also influences the dosing recommendations. If the efficacy studies were performed without any special instructions regarding concomitant food intake, this could be reflected in the text regarding "Dosage and Administration" recommendations. However, it is highly advisable to investigate potential food effects prior to the start of the Phase III program,

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since unidentified or disregarded food effects may jeopardize a positive outcome of the confirmatory efficacy trials.

Dose Proportionality

Dose proportionality, i.e., a proportional increase in exposure (AUC and/or C_{max}) of a drug after a corresponding increase in dose, indicates linear pharmacokinetics of the drug. A higher exposure than predicted from the given dose may indicate saturable metabolism or saturable first-pass effects. A lower exposure than predicted from the given dose may indicate limitations in the absorption processes. Early information on dose proportionality can usually be obtained in the first safety and tolerability study. A more confirmatory study, investigating the intended therapeutic dose range should be performed in an adequate number of subjects and, preferably, with a pharmaceutical formulation that is relevant to the one that will be used in the confirmatory clinical trials in patients. Although the use of an oral solution generates basic pharmacokinetic information regarding the drug substance, choosing an early prototype immediate release formulation or a Phase II/III formulation could give additional valuable information.

Choice of Dose

The dose linearity over the intended therapeutic dose range should be fully investigated, and included in an NDA submission. However, in the early stages of drug development the therapeutic dose range is usually not well established, and therefore it is advisable to investigate the pharmacokinetics of a new chemical entity over a wide, although reasonable, dose range. Especially the upper parts of the dose range is of interest, since the break-point for potentially clinically relevant nonlinearities in the pharmacokinetics of a drug should be captured and quantified as early as possible in the development program.

An adequate number of dose levels (≥ 3) should be examined, but a fixed number of dose levels are not required. It may not be necessary to repeat the dose-linearity study with the to-be-marketed pharmaceutical formulation unless substantial formulation changes have been made, or potential nonlinearities have been identified. However, the reader is referred to Part B: Biopharmaceutics for relevant information regarding waivers and bioequivalence requirements.

Study Population

The study can be performed in healthy, adult male and female volunteers, above 18 years of age. If the intended target population mainly consists of,

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e.g., elderly patients, more valuable information may be generated by performing the study in healthy elderly volunteers or in the target patient population.

Study Design

A single-dose, randomized, crossover design, is the most common choice for a dose-proportionality study. An incomplete block design, where an equal number of subjects are randomized to receive different doses and all cohorts together cover the full range of doses, is also an option. The latter design is occasionally employed when the total blood volume collected from a single volunteer would exceed standard limits of blood donations. The number of subjects participating in the study should be based on earlier studies where intersubject, and if available, intrasubject variabilities have been determined. Study drug should be administered with a standardized volume of water after overnight fast, and standardized meals should not be served until four hours post-dose.

Concomitant food intake should be avoided, unless the drug is associated with adverse events, such as nausea or vomiting, which could be circumvented by a small meal. It is advisable to include the rationale for coadministration of the drug and food in the protocol. If the drug is associated with adverse events that preclude high single doses, a titration design where the pharmacokinetics is determined at steady state can be an alternative.

In certain cases, a parallel-group design may be selected instead of a crossover design, e.g., for drugs with a long terminal half-life, although a substantially larger number of subjects may be needed compared to a crossover design. If a crossover design has been chosen, the investigational periods should be separated by an adequate washout interval ($>5t½$) to ensure that elimination is complete before a second dose is administered. Blood samples should be collected to adequately describe the full plasma/serum drug concentration profile, especially the terminal phase should be adequately described, where sampling should be continued up to at least three to four terminal $t½$ of the drug and/or active metabolites.

Data Analysis

Standard pharmacokinetic parameters (C_{max}, t_{max}, AUQ_t, AUC_∞, CL/F, $t^1/_2$) are calculated by nonparametric or parametric methods for the intact drug and major active metabolite(s). The reader is referred to the section Data Analysis on page 199 of this chapter, for a more detailed description of the

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calculations. The parameters describing exposure (C_{max} and AUC) or apparent oral clearance *(CL/F)* are of most interest for orally administered drugs. For short-acting drugs, such as agents for the treatment of insomnia or acute pain, the intial exposure (truncated AUC up to C_{max} or C_{max}) may be a more relevant descriptor for dose proportionality than AUC_{∞}.

These parameters are graphically displayed vs the administered dose, where a straight line indicates linear pharmacokinetics over the studied dose range. It is recommended that the analysis is performed after dose normalization of the parameters has been performed. There is no formal regualtory recommendations regarding the method of choice. The interested reader can find points to consider regarding the statistical analysis to determine dose proportionality in an article by Gough et al. [22], where a comparison of the performace of different statistical methods was investigated. The data should also be analyzed regarding the similarity of the other pharmacokinetic parameters at the different dose levels, a shift in terminal half-life or t_{max} between doses may need additional attention, and the potential clinical relevance of any dissimilarities in these parameters between different doses should be considered.

REPEATED-DOSE STUDIES

The majority of drugs are intended for chronic or multiple dose therapy in the treatment of a specific medical condition. Even if the pharmacokinetics has been shown to be linear over the intended therapeutic dosing interval after single doses, this may not hold true after repeated dosing. Therefore, the pharmacokinetics of the drug after repeated administration needs to be investigated. Time-dependencies in the pharmacokinetics, such as autoinduction or inhibition of the drug's own metabolism, may occur. A qualitative indicator can be obtained from in vitro studies or preclinical pharmacokinetic studies in animals; however, the magnitude of the potential time-dependency, or lack thereof, can only be assessed in vivo in humans.

Choice of Dose and Dosage Regimen

The pharmacokinetics after repeated administration of the highest dose level in the anticipated therapeutic dose range should be adequately described, since more prominent changes are expected to occur at higher dose levels. It is prudent to include one or two lower dose levels, to fully establish the pharmacokinetic properties of the drug at steady state, after repeated dosing. The pharmaceutical formulation of an oral dosage form

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should preferably be similar to the formulation used in the later clinical trials in the patient population. However, if the results from the single-dose trials call for the development of a modified release or extended release formulation, a smaller trial at an adequate dose level using an immediate release formulation could be considered. If apparent nonlinearities in the steadystate pharmacokinetics of the drug are observed at a later stage, such a pilot study could be used to the differentiate between apparent nonlinearities due to time-dependencies in drug metabolism, and the effects of the altered release profile by the pharmaceutical formulation.

The dosing regimen, i.e., the time-interval between doses, is governed by the exposure (pharmacokinetic)-response (pharmacodynamic) relation-ship of the drug. If relationship is known, the clearance and terminal $t½$ of the drug can be used in the calculations of the optimal-dose regimen [1, 23]. Although the exposure-response relationship may be less well-characterized, the information about the pharmacokinetic properties of the drug will aid the choice of dosage regimen. A drug with a short terminal $t½$ and high clearance, where the desired effect is more likely to be related to the AUC rather than C_{max}, will require more frequent dose intake than a drug with a longer terminal $t½$ and a lower clearance. The reader is also referred to relevant chapters in the Biopharmaceutics section of this book, for pertinent information regarding waivers and bioequivalence studies that may be needed to fulfill all requirements for an NDA, if major changes in the pharmaceutical formulations have been made during the development program.

Study Population

As described elsewhere in this chapter, the study population of choice is usually healthy, adult male and female volunteers, above 18 years of age. The pharmacokinetics of the drug after repeated dosing should also be studied in the intended target patient population, and compared to that of the healthy volunteers. However, the comparison of the steady-state pharmacokinetics of the drug between healthy volunteers and patients can be made across studies, and a direct comparison in the same study is not necessary.

Study Design

An open-label, randomized crossover design is usually chosen if more than one dose-level is included in the study. The number of subjects participating in the study should be based on data regarding variability from earlier studies. Study drug should be administered according to the chosen dosage

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regimen, based on the currently available pharmacokinetic and pharmacodynamic information. Concomitant food and drug intake during the investigational days is usually restricted, and the drug is administered in the fasting state or the drug and food intake is separated by a time-interval of approximately two to four hours.

Blood samples for drug analysis should be collected to adequately describe the attainment of steady state (samples collected immediately prior to the next dose intake during 3–4 dosing intervals, i.e., trough concentrations) and the full plasma/serum drug concentration profile during one, usually the last, dosing interval at steady state. It is recommended that the blood sampling is continued to adequately describe the terminal phase after the last dose intake, e.g., the collection be continued up to at least four terminal $t½$ of the drug and/or active metabolites.

Investigational periods are usually separated by an adequate washout interval ($>5t½$) to ensure that elimination is complete before a second-dose regimen is initiated. Alternate designs, where the subsequent study periods are immediately initiated, without a washout period, should be carefully considered, and only be used if the lack of time-dependent changes in the pharmacokinetics of the drug has been established. An alternate approach is to combine a single-dose and the repeated-dosing regimen in the same subject. In that case, adequate blood sampling should be performed after the first dose, and the repeated dosing is started immediately after the last blood sample of the single-dose period, and blood sampling is performed when steady state has been attained, as described above.

Data Analysis

As described elsewhere, the standard pharmacokinetic parameters (C_{max}, t_{max}, *CL/F*, $t½$; in case of the administration of a single dose: AUC_t, AUC_∞) are usually calculated by nonparametric or parametric methods for the drug and major active metabolite(s). The parameters that are specific for repeated dose administration are the AUC during one dosing interval (AUC_t) at steady state and the accumulation ratio. The latter can be directly calculated if single-dose data also is available. The reader is referred to the section "Data Analysis" on page 199 of this chapter, for a more detailed description of the calculations.

The choice of analysis of the attainment of steady state, from the trough plasma concentrations of the drug, should be stated in the protocol. If more than one dose level is investigated, an analysis of dose proportionality should be performed. It is advisable to include more than one dose, since an unexpected observation of a time-dependency in a parameter, e.g., a larger

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than expected AUC, may not only be caused by metabolic inhibition, but could also be due to pharmacokinetic model misspecification. For example, the terminal $t½$ may not have been correctly determined, potentially due to lack of sensitivity in the analytical method, or the full degree of drug accumulation in tissue has not been previously achieved after single-dose administration. In addition to the analysis of the attainment of steady state (and dose proportionality if applicable), the report should contain clear graphs and tables of both individual and average data, as well as summary statistics.

SUMMARY

In conclusion, a relatively limited number of studies are required to adequately describe the basic pharmacokinetic properties of a drug. Although healthy adult volunteers are usually the population of choice for the basic pharmacokinetic studies of a drug, the validity of the data in comparison with the pharmacokinetics of the drug in the target patient population should also be established. A cross-study comparison with regard to the standard pharmacokinetic parameters (C_{max}, t_{max}, AUC, and $t½$) for one or two dose levels would suffice if the pharmacokinetics are similar in the two populations. The information that has been gathered in the studies described in this chapter is usually included in an NDA submission. In addition to these studies, pharmacokinetic studies in special populations or disease states, drug-drug interactions, and bioequivalence studies, as described elsewhere in this book, are usually included in an NDA submission. Table 1 summarizes the information that is generally expected in an NDA submission.

It can be concluded that the choice of a study design based on careful evaluation of previously gathered data from preclinical and/or prior pharmacokinetic studies is essential to optimize, or minimize, the number of pharmacokinetic studies needed in a development program. Although the timing of the pharmacokinetic studies has not been discussed, the pharmacokinetic information should be used throughout the development program, since a simple description of the pharmacokinetics of a drug serves no purpose in itself. The pharmacokinetic properties should be a valuable instrument in the rational development of the drug. Therefore, it is also vital to include exposure-response analyses of relevant pharmacodynamic parameters throughout the development program, to achieve the best possible knowledge base relevant to the therapeutic use of the drug.

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TABLE 1 Summary of the Descriptive, Basic Pharmacokinetic Information that is Generally Expected in an NDA Submission for a New Chemical Entity

Information	Proposed study design	Population/Route of administration	ICH or FDA Guidance Document
ADME (including metabolic pattern and quantification of major metabolites)	Single doses	Healthy volunteers/ intravenous + oral (or intended route of dosing in patients)	No (Regional regulations regarding isotope studies in humans)
Dose proportionality	Dose escalation	Healthy volunteers/oral (or intended route of dosing in patients)	(Yes, in part) ICH M3 (1st dose in man) Gender: www.fda.gov/ cder
	Single doses Repeated doses		
Bioavailability (absolute or relative)	Single doses	Healthy volunteers/ intravenous + oral (or intended route of dosing in patients)	Yes www.fda.gov/ cder
Drug accumulation	Repeated doses	Healthy volunteers + patients/oral (or intended route of dosing in patients)	No
Food effects	Single doses	Healthy volunteers (or patients)/oral	Yes www.fda.gov/ cder

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10

Surrogate Markers in Drug Development

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INTRODUCTION

PK/PD Relationship

Several conferences and publications starting in the early 1990s until recently have emphasized the crucial role that pharmacokinetic-pharmacodynamic (PK/PD) modeling and the use of surrogate marker can have in streamlining the drug development process [1–9]. In particular, the advent of pharmacogenomics and biotechnology-derived drug products are thought to accelerate and facilitate the use of these techniques in making the drug development process and regulatory decision-making more rational and efficient [5, 8].

PK/PD modeling attempts to establish quantitative (e.g., mathematical and/or statistical) relationships between dosing regimen and pharmacological (PD) responses, and possibly clinical outcomes (see also Chapter 11).

As shown on Fig. 1, PK relates the dosing regimens of the drug product (e.g., dose, dosing interval, rate, and route of administration) with drug or metabolite concentrations in the body, typically measured in plasma. Both

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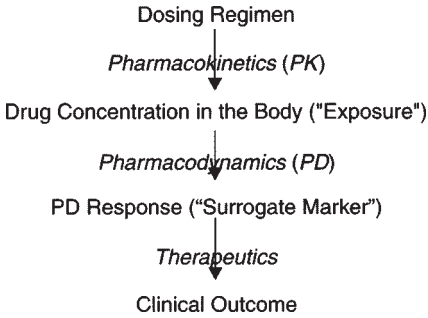

FIGURE 1 Surrogate markers in clinical pharmacology (exposure-response paradigm) and sources of variability.

dosing regimens and/or systemic concentrations are reflective of drug exposure to the patient: The assigned dosing regimen to a patient may reflect nominal exposure, while systemic concentrations (e.g., AUC, Cnax? etc.) reflect systemic exposure. The latter exposure measure is more closely related to drug/metabolite concentrations at the receptor site(s) responsible for the drug-induced pharmacological effect(s). It also allows to compare patients based on variability in medication adherence (compliance), as well as drug absorption and disposition that may be affected by patient covariates and contribute to the overall variability in drug response (see Chapters 8 and 9).

On the other hand, PD relates the drug concentrations in the body to any observable (multivariate) pharmacological response. A pharmacological response can be any physiological, biochemical, or pharmacogenomic endpoint that can be measured and is temporally and causally related to the drug. This PK/PD relationship is also referred to as the exposure-response (ER) relationship. Any variability in this relationship within and between patients contributes to the overall variability in drug response. In general, the PD responses are mediated by the mechanism(s) of action (MOA) of the drug. Nevertheless, a drug may have additional PD effects that are not mediated by the primary MOA such as hepatotoxicity.

Finally, the PD response(s) may be related to the ultimate clinical outcome(s), i.e., clinical efficacy and toxicity. If so, these (special) PD responses are surrogate markers that may substitute for clinical outcomes, since they usually are easier to measure and allow appropriate dosingregimen adjustments without having to accept adverse clinical outcomes.

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It is one of the basic tenets of clinical pharmacology that an exposure-response relationship exists for clinical outcomes; namely, that changing the dose, etc., (exposure) has a tangible impact on outcomes. As a corollary, it is essential to optimize the dosing regimen according to the known PK/PD covariates.

Surrogate Markers

The choice of the term "marker" used to indicate a marker of biological drug response (biomarker) or the clinical outcomes (surrogate marker) originates from clinical medicine, where markers are used to indicate absence or presence of a disease (diagnostic purpose) and/or predict the rate and extent of disease progression (prognostic purpose).

As shown in Fig. 2, these markers are strongly tied to our understanding of the pathophysiology of the disease (POD) being treated. The use of markers in clinical medicine for diagnostic or prognostic purposes is justified based on epidemiological and/or interventional clinical studies that assess their ability to predict clinical outcomes.

Dosing-Regimen Optimization

Using the PK/PD framework discussed above along with the use of surrogate markers allows the optimization of dosing regimen in the drug development and in clinical practice.

Figure 3 illustrates typical exposure-response relationships for clinical efficacy and toxicity. Depicted is the percentage of patient responding (i.e., showing either efficacy or toxicity) as function of an exposure measure. In the simple case, these relationships can be thought of as dose-response curves for efficacy and toxicity. Both exposure-response curves show a sigmoidal relationship due to the above mentioned population variability in PK and PD. An optimal exposure (e.g., dose) is designed to minimize the likelihood of toxicity while maximizing the likelihood of clinical efficacy.

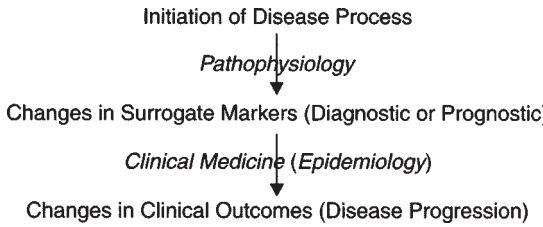
Initiation of Disease Process
Pathophysiology
Changes in Surrogate Markers (Diagnostic or Prognostic)
Clinical Medicine (Epidemiology)
Changes in Clinical Outcomes (Disease Progression)

FIGURE 2 Surrogate markers in clinical medicine (epidemiology).

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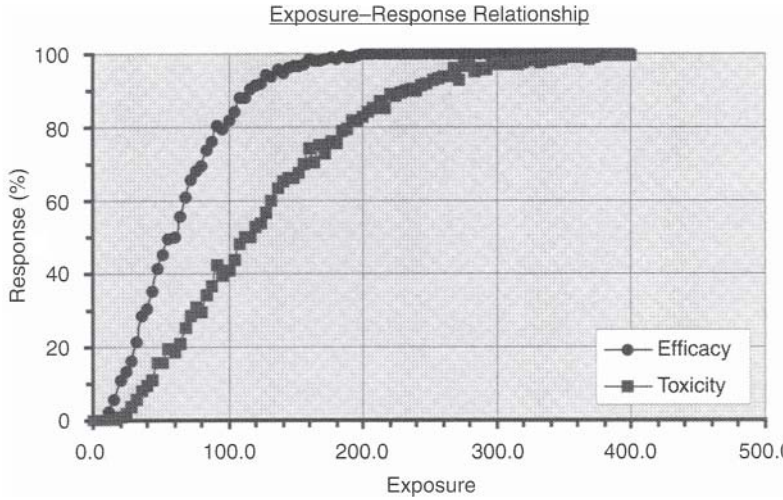

FIGURE 3 Example of exposure-response relationships for clinical efficacy and toxicity.

Knowledge of this ER relationship allows the rational selection of an optimal exposure. Note that similar relationships are expected to exist for biomarkers and surrogate markers as well, but their shape and variability may be quite different.

Therefore, it is essential during the early clinical drug development process to identify important clinical covariates (such as age, gender, renal function, comedications, etc.) of the PK and PD drug properties, along with a potential surrogate marker of efficacy and/or toxicity. The former information allows the rational selection of a patient-specific dosing regimen (dose individualization) while the latter allows continuous assessment of the therapeutic regimen and can trigger dosing regimen adjustments intended to avoid toxicity and/or improve efficacy.

In clinical practice, using the above information provided on the approved drug-product label permits the prescriber to individualize the patient-treatment regimen and to continuously monitor the treatment success using the surrogate marker (therapeutic drug monitoring, TDM). This is particularly important for diseases and drugs where the ultimate clinical outcome is mortality, and a suboptimal dosing regimen is likely to result in excess mortality due to either lack of efficacy or toxicity. Table 1 is an incomplete list of some biomarkers/surrogate markers used in drug development and clinical practice (see also Chapter 12).

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TABLE 1 Examples of Bio-/Surrogate Markers and their Basis in POD and/or MOA (see text for abbreviations)

Bio-/surrogate marker	Disease/drug treatment
QT_c-interval	Fatal arrhythmias (numerous drugs, long QT_c-interval phenotype) POD, MOA
Cholesterol, blood pressure	Cardiovascular disease progression (numerous drugs, numerous diseases) POD, MOA
FEV_1, PEF	Chronic asthma (disease progression, numerous drugs) POD, MOA
CD_4 count, HIV viral load	HIV infection (numerous drugs, disease progression) POD, MOA
CsA serum concentration	Prevention of organ rejection (CsA therapy, TDM) MOA
INR (PT)	Prevention of strokes (warfarin therapy, TDM) MOA, POD
Hematcocrit	Chronic renal/cancer anemia (erythropoeitin therapy, TDM) MOA

The QT_c-interval (measured on the electrocardiogram) has been shown to predict the occurrence of fatal arrhythmias (torsades de pointes, TdP) associated with quite a few drugs, some of which have recently been withdrawn from the marketplace due to insufficient risk/benefit ratio (namely, terfenadine, astimazole, etc.). Prolongation of the QT_c-interval is thought to be a precursor of TdP.

Plasma cholesterol (a biochemical measure) and blood pressure (a physiological measure) are some of the oldest surrogate markers. Initially, during epidemiological studies in the 1960s (Framingham), elevated levels of these markers were shown to be associated with increased cardiovascular mortality and morbidity. Later on, in prospective interventional clinical studies using blood pressure or cholesterol-lowering medications and diets, the markers were shown to be causally related to cardiovascular outcomes. Additionally, mechanistic studies elucidated the POD, i.e., the pathophysiological chain of events leading from hypercholesterolemia and hypertension to cardiovascular morbidity and mortality.

Pulmonary function tests such as FEV1 and PEF are used in clinical practice to assess the progression of chronic bronchial asthma as well as to monitor treatment with steroids and bronchodilators, to change drug and/or dose, if necessary.

The CD_4-lymphocyte count in peripheral blood was the first surrogate marker used in the marketing approval of AZT (zidovudin) for the

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treatment of HIV infection in the late 1980s. At that time, higher CD_4 counts were found to be negatively correlated with disease mortality. Mechanistic studies had shown the role of CD_4 lymphocytes in the pathophysiology of HIV infection. Due to the poor prognosis of the disease and the lack of any effective treatment, AZT was approved based on clinically significant increases in CD_4 counts rather than a proven mortality benefit, which was shown later in phase IV studies. Currently, the HIV viral load in plasma is the accepted surrogate marker of disease progression and treatment success, both in drug development and clinical practice. In the future, HIV pheno-/genotyping may be an even better predictor of clinical outcomes.

Cyclosporine (CsA), used to prevent organ rejection, is known to have a high level of between- and within-patient PK variability, and the consequences (clinical outcomes) of inappropriate exposures are severe, namely organ rejection (lack of efficacy) or renal toxicity. As a result, CsA serum concentrations are measured (as a surrogate endpoint) and used to adjust the dosing regimen, if necessary.

The International Normalized Ratio (INR), an in vitro coagulation test is used successfully in the TDM of warfarin therapy, an oral anticoagulant. Warfarin is known to be associated with high PK and PD variability between and within patients; the consequences of inadvertently low or high exposure of warfarin can be disastrous, namely ischemic or hemorrhagic stroke. INR values have been shown to predict these clinical outcomes, and target INR ranges have been established to guide warfarin dosing. It is noteworthy to recognize that the INR predicts both efficacy and toxicity since both outcomes are due to the MOA of warfarin.

Finally, blood hematocrit is used as a surrogate marker in the treatment with erythropoietin (epo) since it does predict quality of life, and epo is a very expensive treatment mandating appropriate dose selection and adjustments in clinical practice.

DEFINITIONS

Consensus has been reached on the terminology of the different markers [6, 7, 9]:

Terminology of Markers

1. *Biomarker (Intermediate Endpoint):* A biological (pathophysiological or pharmacological) indicator that can be measured as a result of a therapeutic intervention. It may or may not be related to clinical outcomes, but is involved in the chain of events in the POD and/or MOA the drug.

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2. *Clinical Outcome:* A clinically accepted indicator of disease state/progression, e.g., survival, morbidity, symptom scores, etc. Clinical outcomes are measures of the efficacy or safety/toxicity of a drug.

3. *Surrogate Endpoint/Marker:* A biomarker that predicts clinical outcomes as accepted by the scientific, medical and regulatory community. It may substitute for clinical outcomes in the drug development process (dosing-regimen and dosage-form optimization and possibly drug approval) and in clinical medicine (TDM). At least some of the variability in clinical outcomes is explained by changes in surrogate markers. [6, 7]

A biomarker (candidate/putative surrogate endpoint in the drug discovery/development process) can achieve surrogate endpoint status if properly evaluated. Evidence to support that linkage integrates information from multiple sources such as molecular biology, pathophysiology of the disease, mechanism of action of the drug candidate, clinical trials, and epidemiological studies.

EXPOSURE-RESPONSE RELATIONSHIP

The exposure-response relationship measures the association between responses (clinical outcomes, surrogate markers, biomarkers) and drug exposure (dose, systemic concentrations, etc.). This relationship can be modified by clinical covariates, both intrinsic and extrinsic. Clinical pharmacology studies help in elaborating the shape and variability of this relationship (see Chapter 11).

Characteristics of Markers

Based on the measurement scale that they are measured on, PD markers can be classified as follows:

1. *Graded Response:* A quantifiable PD marker (such as an in vivo physiological response or in vitro test) that is causally and temporally linked to drug treatment and related to drug exposure (ER relationship), e.g., blood pressure, serum cholesterol, INR, etc. These endpoints are usually chosen based on the MOA of the drug and known receptor-mediated physiological or biochemical responses.

A graded response is a continuously scaled variable, can be measured repeatedly within the same individual, and is typically used for PK/PD modeling, particularly preclinically and in phase I/II.

2. *Challenge Response:* A quantifiable, graded response to a standardized exogenous challenge agent that is modified by administration of the drug of interest and related to drug exposure, e.g., exercise-induced tachycardia (to assess β_1-blocker activity), and histamine-induced broncho-constriction (to

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assess H_1-blocker activity). These markers are based on the MOA of the drug and sometimes on the POD. This kind of markers usually requires additional special clinical testing and are rarely used in clinical practice for dose adjustment.

A challenge response is a continuous variable (e.g., percent inhibition relative to baseline or placebo). It requires additional interventions, may not be repeated often within the same individual during a dosing interval, and contributes possibly unacceptable additional safety issues in phase I/II studies. However, it can be used for PK/PD modeling.

3. *Categorical Response:* A "Yes-or-No" response due to drug administration that can be related to drug exposure, e.g., death, organ rejection, incidence of AE. This type of response is usually a clinically relevant outcome based on the disease progression in question, regardless of the MOA. It can be measured as part of clinical practice, but does not allow treatment adjustment.

However, it can be measured only once within a given patient. It is a nominal variable that is not very informative statistically and requires a large sample size. It is used in phase II/III studies along with population PK/PD analysis.

4. *Time-to-event Response:* Time-to-event that is related to drug exposure, e.g., survival time, time to relapse. This type of response is usually a clinically relevant outcome based on the disease progression in question, regardless of the MOA. It can be measured as part of clinical practice, but does not allow treatment adjustment.

It is a censored continuous variable that can be measured only once within a patient, is not very informative, and requires a large sample size in phase II/III studies along with population PK/PD analysis.

5. *Event Frequency/Rate Response:* Frequency of clinical events related to drug exposure, e.g., seizure frequency, frequency of cardiac arrhythmias.

It is a censored continuous variable that can be measured more than once within a patient; however, is not very informative, and requires a large sample size in phase II/III studies and population PK/PD analysis.

USE AND BENEFITS IN DRUG DEVELOPMENT

Markers in Drug Discovery and Development

Biomarkers have to be identified early during the drug-discovery process and evaluated/validated systematically throughout the subsequent drug-development process:

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1. *Discovery:* Potential biomarkers/surrogate makers should be selected based on the current mechanistic understanding of the pathophysiology of the disease and proposed mechanism of action of the drug candidate based on theoretical considerations and/or experimental evidence. Additional thought should be given to potential toxicity markers not associated with the known MO A (e.g., other drugs in the same pharmacological class, known toxicities in disease, known biomarkers in the disease).

2. *Preclinical Development:* The in vitro binding of the drug candidate to receptor/enzyme and/or in vivo or ex vivo functional testing (enzyme activity or receptor intrinsic activity) should be evaluated for feasibility as markers across various species, including humans. Ex vivo or in vivo challenge paradigms based on the MOA should be considered. As part of the preclinical workup, ER relationships for potential markers of efficacy/toxicity should be established. This will allow interspecies scaling and optimal selection of starting dose and dose-escalation increment or even a PD-guided study design for phase I for the first time in human studies.

3. *Clinical Development:* In phase I, in vivo testing/challenge paradigms in healthy volunteers should be considered to establish the ER relationship in low-population-variability setting. In phase II, demonstration of changes of biomarkers in the expected direction may serve as proof-of-concept (POC) suggesting clinical efficacy of the drug candidate, and help in making important Go-No Go decisions. Throughout the phase II stage, biomarkers should be correlated with short-term clinical outcomes in the target patient population; attempts should be made to establish ER relationships for biomarkers/short-term clinical outcomes. This correlation between biomarker and accepted (approvable) clinical outcomes should be quantitated in the phase III program, and important clinical covariates affecting outcome and marker should be identified. If necessary, the surrogate marker can be used for therapeutic monitoring of postmarketing in clinical practice. Demonstrated ER relationships with biomarkers or surrogate markers will also be useful in phase IV to assess new dosing regimens, dosage forms, and special populations (namely pediatrics).

Benefit of Using Markers in the Drug Development Process

1. *Identification of Biological Sources of Variability in Drug Response:* For rational drug development, it is important to understand the contribution of PK or PD variability to the overall population variability in drug response (for dose individualization and TDM).

2. *Physiological Interpretation of PK/PD Parameters:* Appropriate physiological interpretation of PK and PK/PD parameters early in the drug

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development (in-vitro, preclinical, phase I) allows appropriate interspecies scaling and informative "Go-No Go" decisions [8].

3. *Identification of Relevant PK/PD Covariates:* The PK/PD approach throughout drug development assists in anticipating and identifying important patient factors, e.g., age, gender, concurrent diseases, and comedications, that may require dose individualization/therapeutic monitoring in the target population (see Chapters 8 and 9).

4. *Rational Optimization of Dosage Forms and Dosage Regimens:* Understanding of the intrinsic PK/PD characteristics with an acceptable biomarker and sources of population variability permits better design of dose-finding studies in phase I and II as well as rational development of appropriate dosage forms. It may also be useful in selecting optimal backup compounds to the lead compound.

5. *Rational Labeling Decisions:* Appropriate PK/PD modeling with an acceptable biomarker helps assessing and interpreting the PK results of "equivalence" studies, i.e., food-effect, chronic renal and hepatic disease-effect, and drug-drug interaction studies, by allowing to define a target range of "no clinically significant PK difference" ("What-If" Scenarios).

It is the PK/PD information gained in the drug development process that drives the final clinical dosing-regimen recommendations (particularly dose individualization and therapeutic monitoring) in the product label.

6. *Marketing Approval:* PK/PD studies with an acceptable surrogate marker may provide supportive ("confirmatory") evidence for drug approval in lieu of an (second) adequate and well-controlled phase III clinical trial, particularly for extension into special populations (e.g., pediatrics) or new dosage forms. However, this is likely to occur only if the surrogate marker has been accepted after comprehensive evaluation and other drugs in the same class have shown benefits in clinical outcomes.

Assessment of Measurement Performance of Biomarkers

In addition to their validity, the measurement techniques for biomarkers and surrogate markers have to be assessed for their reliability in practice [6, 10]:

1. *Sensitivity:* The ability of the of the measurement technique to detect small changes in the marker.

2. *Specificity:* The ability of the measurement technique to differentiate drug-induced changes from spontaneous changes in the marker.

3. *Reproducibility (Accuracy and Precision):* The ability of the measurement technique to provide consistent results throughout clinical studies and development programs.

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Tests used in clinical practice may not necessarily be rigorous and rugged enough to measure biomarkers and surrogate markers as part of a drug development. Additional technology may be needed to improve the reliability of the measurement techniques.

Integration of Knowledge Gained during Development Process

PK/PD modeling with biomarkers and/or surrogate markers use and combine quantitative information from various disciplines such as pharmacology, toxicology, pathophysiology, clinical pharmacology, and biopharmaceutics. This allows each discipline to provide important input in each phase of the drug development. Throughout the development, information will need to updated, PK/PD models revised, PK/PD model parameters adjusted, and biomarkers evaluated for their further use.

If done consistently, the PK/PD database can serve as the foundation of clinical trials simulations (see Chapter 11). Clinical trials simulation uses PK/PD models and model parameters (and their statistical distributions) to predict clinical outcomes as function of dosing regimens or study designs. This is extremely useful in optimizing clinical study designs and sample size for phase II/III studies.

LIMITATIONS

Limitations of PK/PD Modeling using Surrogate Markers

1. *Validation/Evaluation of Surrogate Endpoints:* What is the relationship between changes in (surrogate) PK/PD endpoints and clinical acceptable efficacy and/or safety outcomes? The validity of PK/PD modeling depends on both surrogate endpoint validation and PK/PD model validation. Surrogate endpoint validation is a continuous process that should start at the preclinical stage; it requires front-loading of the drug-development process.

2. *Incorporation of Long-term Disease Progression and Subpopulations:* If the PD endpoint is clinically meaningful (surrogate marker), the effect of disease progression in patients with the disease may have to be incorporated as baseline PD model in the PK/PD model. If possible, the endpoint should be demonstrated to be meaningful across subpopulations of patients.

3. *Long-term Changes in PK or PK/PD Relationship (Time-invariance):* Typically, the PK model and the population parameter estimates are obtained from single-dose or short repeated-dose studies, which do not

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reflect the reality of chronic treatment of most chronic diseases. However, the PK may change over time, e.g., due to autoinduction or other secondary drug-induced changes in PK.

Typically, the intrinsic ER relationship (e.g., effect-biophase concentration relationship) is assumed stationary, i.e., invariant with time [10]. This means that at (PK and PD) steady state, there is a constant relationship between effect and plasma concentration. However, there is an increasing number of drugs where this is not necessarily true, and PD tolerance or resistance develops as function of time and dosing regimen, and the "intrinsic" PK/PD relationship changes with time.

4. *Empirical vs. Mechanistic PK/PD Modeling:* The objective of the PK/PD modeling exercise determines the use and validation of PK/PD models: Empirical models may be validated for their predictive ability, but do not allow interpretation of their model parameters (if parametric), i.e., the system is considered a "black box". On the other hand, mechanistic models allow estimation of meaningful PK/PD parameters, but the data obtained from typical clinical studies may prevent accurate and precise parameter estimation.

5. *PK/PD Model Validation:* PK/PD model validation is a clinical pharmacology issue based on statistical concepts. However, internal model validation is only a part of PK/PD model validation: The surrogate PD endpoint used has to be clinically validated (external validation), i.e., has to be linked to clinically acceptable efficacy or safety outcomes (accepted/approved by the medical specialists). There is growing research activity attempting to link surrogate PD endpoints (typically continuously scaled variables) mathematically to clinically relevant outcomes (typically categorical variables), as shown in clinical trials simulations (e.g., QT_c-prolongation and likelihood of TdP).

Any PK/PD model, be it empiric or mechanistic, parametric or nonparametric, can and has to be validated for its intended use: Validation means assessment of descriptive performance (interpolation), predictive performance (extrapolation), and estimation of meaningful PK and PK/PD parameters that can be interpreted. In general, the PK/PD models have to be predictive (within certain constraints of dosing regimens and time) to be useful, but not necessarily mechanistically interpret able.

Potential Pitfalls of Surrogate Markers

Since surrogate markers are expected to substitute for clinical outcomes, the following situations may occur:

1. *Perfect Surrogate Endpoint:* The full effect of the (drug) intervention on clinical outcome(s) is reflected and predicted by corresponding changes in the marker (perfect correlation). This ideal

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scenario does not exist (yet), and probably never will, since no single marker can reflect the entire (multivariate) pathophysiology of a disease or pharmacology of a drug [8].

2. *Acceptable Surrogate Endpoint:* Changes in the marker reflect only partially the (drug) intervention effect on clinical outcomes, e.g., cholesterol for statin drugs, blood pressure for antihypertensives, HbA_{1c} for antidiabetics, etc. These are endpoints that, based on available evidence, are accepted by the scientific and medical community to substitute for clinical outcomes, both in the drug development and in clinical practice.

3. *False Positive Endpoint:* The drug intervention affects the marker favorably, but has an unfavorable effect on clinical outcome, e.g., premature ventricular contraction (PVC) frequency for antiarrhythmic agents: The placebo-controlled, randomized, double-blind Cardiac Arrhythmia Suppression trial (CAST) demonstrated that various antiarrhythmic agents did suppress PVC frequency in patients with cardiac arrhythmia, which had been thought to predict improved clinical outcome, namely mortality. However, CAST showed excess mortality in the active-treatment groups relative to the placebo group (most likely due to the arrhythmogenic effects of the drugs), disproving PVC suppression as a surrogate marker.

From a regulatory point of view, this appears to be the major concern in using surrogate endpoints to approve drug products for marketing, and necessitates the requirement of adequate and well-controlled clinical phase III trials to demonstrate efficacy.

4. *False Negative Endpoint:* The drug intervention affects the marker unfavorably (or not at all) but has a favorable effect on clinical outcomes, e.g., Prostate-specific antigen (PSA) in treatment of prostate cancer.

Evaluation/Validation of Surrogate Endpoints

Evaluation or validation of biomarkers to serve as candidate surrogate markers is an ongoing process starting in the drug-discovery stage and continuing throughout the drug-development process. The extent of validation depends on the intended use of the marker; e.g., if the surrogate marker is intended to be used for drug approval (in lieu of clinical evidence of efficacy or toxicity), there is a high burden of evidence to that effect. On the other hand, if the biomarker is used for internal decision-making, such as Go-No Go after POC or other phase I/II studies or dose selection for phase II/III studies, less evidence to support their use is necessary. Evidence to support the contention that a biomarker may be a surrogate for clinical outcomes can be derived from the following studies:

1. *Mechanistic studies* identify the biomarker(s) based on our knowledge of the pathophysiology of the disease and the mechanism of action of the

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drug for its efficacy. However, in general, our incomplete understanding of POD and MOA makes this level of evidence the weakest. Furthermore, clinical toxicities may have different (unknown) MOAs unrelated to the MOA involved in clinical efficacy.

2. *Epidemiological studies* demonstrate a correlation (not causation) between biomarker and clinical outcome. These studies are typically designed to stratify patients based on their risk of disease progression and demonstrate the diagnostic and/or prognostic use of markers, typically based on our understanding of the POD.

3. *Clinical pharmacology studies* establish a (temporal and causal) relationship between biomarker and drug administration (ER relationship). This is strong evidence that the drug treatment (rather than other extrinsic covariates) is responsible for the biomarker changes. In conjunction with 1 and 2, clinical pharmacology studies strengthen the validity of a biomarker as a surrogate marker.

4. *Clinical intervention trials* (with the gold standard of a prospective randomized clinical trial) demonstrate a (causal) link between changes in the biomarker and clinical outcomes. This helps establish at least the (partial) predictability of clinical outcomes from the biomarker and allows the biomarker to achieve surrogate endpoint status.

CONCLUSIONS

The impact of PK/PD modeling on the clinical development process and its acceptance by the scientific and regulatory community depends on the acceptance of appropriate surrogate endpoints and the validity of the modeling practice.

Due to our incomplete understanding of pathophysiology of most diseases and mechanism of action for efficacy of drugs, the use of surrogate endpoints may be limited, particularly as markers of toxicity (e.g., hepatotoxicity).

Evaluation of candidate surrogate endpoints has to start early in drug discovery and continue throughout the preclinical and clinical development; it requires additional resources and commitment to interdisciplinary collaboration.

The potential payoff of PK/PD modeling using surrogate endpoints lies in the streamlining of the clinical development and regulatory approval process, and improved therapeutic labeling and monitoring in clinical practice. The approach may also provide supportive evidence of efficacy and/or safety to allow marketing approval under special circumstances (e.g., dosage form changes, pediatric population etc.).

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11

Population Pharmacokinetic and Pharmacodynamic Analysis

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INTRODUCTION

One of the critical objectives of clinical pharmacology is to individualize the dosing recommendations by estimating the population characteristics, for instance the central tendency and the variability, of the fundamental pharmacokinetics (PK) and pharmacodynamic (PD) parameters in the target population. Individualization of dosage includes describing the variability in the PK and PD parameters using covariates such as body weight, age, gender, disease state, concomitant medication(s), etc. In addition, the regulatory agencies and the pharmaceutical drug sponsors use population PK/PD analyses for a variety of other purposes through the drug development process. These include drug candidate selection, dose selection, clinical trial design, gaining insights into clinical trial outcomes and others.

The U.S. Food and Drug Administration (FDA) utilizes population analyses as an aid in making regulatory decisions at almost all stages of the investigational new drug (IND) and new drug application (NDA) review processes. The leadership of the FDA in making the current drug

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development process more efficient is reflected in the many guidances that are issued for industry to date. The FDA is the first institution to set up a pharmacometrics group exclusively for the purpose of reviewing and conducting research in PK/PD modeling and simulation (M&S) related topics. The aim of this chapter is to briefly present the population analyses methods and discuss some specific applications of the same in regulatory review processes.

DATA AND DESIGN

Clinical trial designs dictate the data collection and analysis methods. Every clinical trial is conducted to answer a set of questions. Clinical trial protocols explicitly state how, when, and what to measure in a given individual in order to analyze the data in a prespecified manner. Hence, the analysis plan is an integral part of the experimental design. There are two broad types of data that could be collected in clinical trials—experimental and observational. Many PK/PD measurements are typically collected from a clinical trial that is conducted only in a small number of subjects over a relatively short duration of time. Data from such studies are called as "experimental data." Studies performed to evaluate the effect of food, renal/hepatic impairment, or gender on the pharmacokinetics of a drug (but not part of a large trial evaluating the clinical effect of the drug) are trials where experimental data (10 to 20 samples per individual) are collected. Data from each of the subjects can be analyzed independent (in most cases) of the others and summarized.

On the contrary, when the objective of the trial is to evaluate the effectiveness and safety of a drug in a large number of patients, obtaining 10 to 20 samples per subject may be impossible. But, a few measurements can be performed as part of the routine examination of each of the patients. These measurements are called as observational data. It is almost impossible to analyze the data from each patient separately. Some of the reasons include repeated measures, imbalance, and confounding correlation between the design and outcome [1]. Pharmacokinetic information without adequate understanding of the pharmacodynamics of a drug is futile. The design of the large clinical trials that probe into the pharmacological actions of the drug, hence, needs some discussion.

Although there are several types of designs used to evaluate effectiveness and safety of a drug, the most widely used designs include—parallel, cross-over, and titration. In a parallel design trial, patients are randomized into cohorts who receive one of the several treatments (control, dose 1, dose 2, or dose 3). Such a design will offer the population, rather than the individual,

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PK/PD characteristics. The advantage of such a design is the lack of confounding factors such as time (carry over effects) and design dependent outcomes.

According to a cross-over design, each patient would receive all the possible treatments. Therefore, a cross-over design is the most powerful design if deducing the individual concentration (or dose)-response curves is the ultimate aim. The disadvantages of this approach are that of its longer trial duration, possible carry-over effects from previous doses, and the need for sophisticated data analysis (nonlinear mixed effects modeling).

The titration design ensures that the patients usually start at a relatively low dose and the dose is increased gradually until either no additional benefit is observed or dose-limiting toxicity occurs. This design closely resembles the clinical practice and the individual PK/PD character-istics can be obtained. The major disadvantage of this design is that of the possibility of an inverted U-shaped PK/PD relationship, as an artifact. The patients who are less sensitive to the drug need higher doses of the drug, making it appear as if the response decreases after a certain dose. Data analysis using conventional methods such as ANOVA fails and the use of sophisticated modeling techniques is required.

The control group consists of either active treatment(s) or a placebo, depending upon the type of disease. Where administering a placebo is considered unethical (for example, AIDS trials) active treatment serves as the control group. The trial subjects could be randomized to dose, drug concentration, or effect elicited by the test drug. The trials are, thus, called as randomized dose controlled (RDCT), randomized concentration controlled (RCCT), and randomized effect controlled (RECT) trials, respectively. The RDCTs are the most prevalent due to the relative ease of executing a trial. The test dose(s) are randomly administered to the subjects and data are collected throughout the trial. The so-collected data are then analyzed using an appropriate method (see the following section). In an RCCT [2], the subjects are randomized to a set of prespecified (usually plasma) concentration levels. These target concentration levels are selected based on the PK/PD relationship characterized in previous trials/ experiments. The RCCT requires a dose-titration period where the dose to ensure that the concentrations lie within a target range (e.g., 5 ± 0.5 μg/L) is identified. The requirements to conduct such a trial include: (1) availability of prior information to select the appropriate target concentration ranges, (2) availability of an efficient and sensitive analytical assay method with a short turnaround time, and (3) availability of enough strengths of the formulation to allow for the necessary dose adjustments. In an RECT [3], the subjects are randomly assigned to a set of prespecified target effect levels. Based upon the prior knowledge about the drug's PK/PD, sampling is conducted and the dose

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is adjusted accordingly. The requirements to conduct such a trial are similar to that of RCCT except that in an RECT the effect is targeted. Drugs whose PK have a large unexplained variability are candidates for RCCT and drugs whose PD have a large unexplained variability could be candidates for RECT. When the measured effect (desired/undesired) is symptomatic (those which are "felt" by the patients, e.g., pain, nausea, etc.), RECT could be applicable. When the symptoms are not obvious, RCCT could be a better choice. Unfortunately, there are fewer drug development plans that utilized RCCT or RECT designs.

POPULATION ANALYSIS METHODS

Types of Models

First, an attempt will be made to define a few widely used terms that are needed for the clarity of discussions. All PK (or exposure)/PD (or response) models are made up of several components or sub-models. While "PK" need not be defined, "PD" encompasses drug activity (both desired and undesired effects) as measured by biomarker(s), surrogate(s), and/or clinical end points. The PK/PD sub-models, by and large, can be classified based on their function (descriptive and predictive) and principle (mechanistic and empirical).

Descriptive (Sub) Model. A model or a sub-model whose representation, essentially, confines its use to the range of dependent variable(s) used to build the (sub-) model. Example: A linear concentration-effect relationship may not be able to extrapolate beyond the range of concentrations studied.

Predictive (Sub) Model. A model or a sub-model whose representation allows its use to "predict" within and beyond the range of dependent variable(s) used to build the (sub-) model. Example: An Emax type concentration-effect relationship can be used to extrapolate beyond the range of concentrations studied.

Mechanistic (Sub) Model. A model or a sub-model whose structure and parameterization allow direct and/or indirect linkage to physiological processes. Example: An allometric equation to relate body weight and the clearance of a drug.

Empirical (Sub) Model. A model or a sub-model whose structure and parameterization allow no direct and/or indirect linkage to physiological processes. Example: A linear model to relate body weight and the clearance of a drug.

We note the overlap in the definitions to differentiate models based on function and principle. But there may be cases when a model is empirical

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(mechanistic) in principle but predictive (descriptive) in function. An example would be that of the dual cosine function used to describe the circadian rhythm in most biological processes. Most known models have a combination of the different sub-models.

Basic Framework

The hierarchy in the population analyses is—population (fixed effects), individual (random effects), and then each observation (residual error). A complete population PK/PD model usually constitutes of four structural and three statistical (error) models. The four structural models include: (1) PK model, (2) disease progression model, (3) PD model, and (4) covariate (or prognostic factor) model. The parameters of these models are called as "fixed effects." Examples of fixed effects include the typical value of systemic clearance in a 70-kg person and the mean potency of the drug. The three statistical models include: (1) inter-individual variability (IIV) model, (2) inter-occasion variability (IOC) model, and (3) residual error model. The parameters of the IIV/IOC model are called as "random effects." The random effects models assume that the inter-individual errors *(η)* are distributed with a mean zero and a variance ω^2. The residual error model assumed that the measurement (and model mis-specification) errors are distributed with a mean zero and a variance σ^2. Nonlinear "mixed" effects models deal with both fixed and random effects simultaneously, hence the name.

The framework of the mixed effects models is illustrated in Fig. 1. Consider a one-compartment model when the drug was given as an intravenous bolus. Let us also assume that the volume of distribution *(V)* is identical in every individual (no inter-individual variability). The concentration in the "*i*th" subject at the "*j*th" time point can be described using the following equations:

$$C_{ij} = \frac{\text{Dose}}{V} \cdot e^{(CL_i/V)t} + \varepsilon_{ij} \quad (1)$$

$$\text{CLi—CLPoP} + \eta_{\text{CL},i} \quad (2)$$

Where CL_i is the estimated clearance of the "*i*th" subject, CL_{POP} is the estimated population mean clearance, $\eta_{\text{CL},i}$ is the difference between the population and individual clearances, and ε_{ij} is the residual error of the "*j*th" sample of the "*i*th" subject. The ηCL values are assumed to follow a normal distribution with a mean zero and variance ω^2_{CL}. The εij values are assumed to follow a normal distribution with a mean zero and variance σ^2.

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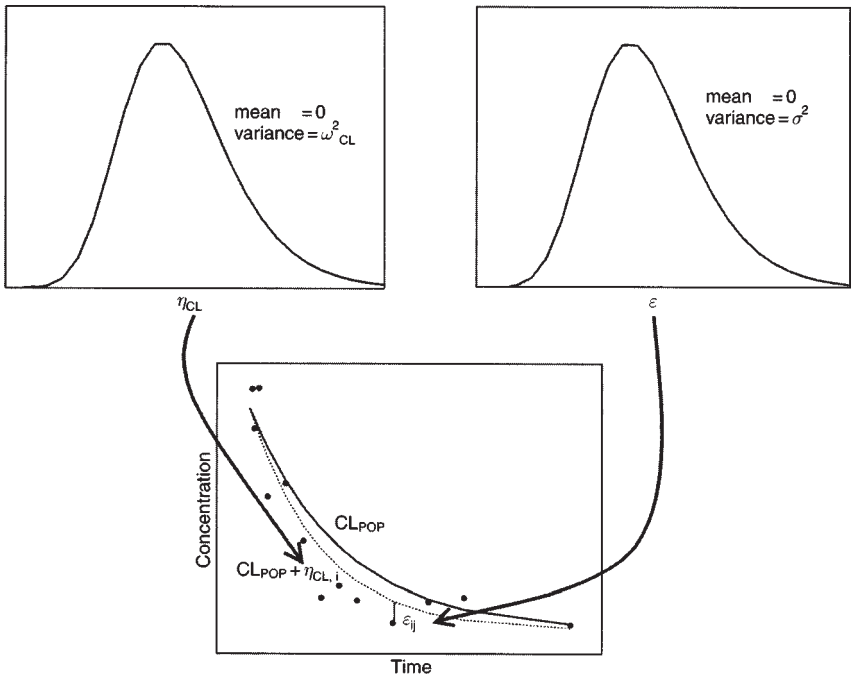

FIGURE 1 The basic framework of nonlinear mixed effects modeling. Consider the "ith" observation in the "*i*th" subject. The difference between the observed concentration (solid circle) and the individual predicted concentration (broken line) is due to the fact that the "ith" individual's clearance (CLi) is different from the population clearance (CL_{POP}) by a value of $\eta_{CL,i}$. An additional source of variability is the residual error (ε_{ij}) which is primarily due to model mis-specification and measurement error. The ηCL values follow a normal distribution with a mean zero and variance ω^2_{CL}. The ei_j values follows a normal distribution with a mean zero and variance σ^2. According to the present example, the NM model would estimate the parameters—CL_{PO}R ω^2_{CL}, and σ^2.

Of the several population analyses techniques, the most popular are: (1) naïve pooled analysis, (2) two-stage analysis (TS), and (3) nonlinear mixed effects analysis (NM). The naïve pooled analysis is performed by pooling data from all subjects (as if all the data are from a single "giant" subject). A minor variation of this method involves analysis of the mean data. Both the methods provide only the central tendency of the model parameters and no random effects are estimated. These methods are applied more routinely when dealing with preclinical data. Naive pooled analysis is appealing because of its simplicity. No sophisticated software is required. The fact that

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the random effects cannot be estimated and inter-individual variability cannot be accounted using covariates (such as body size, age, etc.) makes the potential of naïve pooled data modeling very limited.

The TS method is a reasonably powerful method to estimate both the central tendency and inter-individual variability. The first stage involves the estimation of the individual parameters and the second stage involves the estimation of the population mean and variance of the parameters, after adjusting for covariates if necessary. The TS method requires that enough number of samples (greater than the number of model parameters) per subject are collected, as is the typical case with experimental data. This method assumes that the individual parameters, estimated in stage one, are the true values for the calculations in stage two. By and large, this is a relatively minor concern. The more serious drawbacks include modeling sparse data from observational studies and modeling concentration (or dose)-dependent nonlinear processes. Consider a drug whose elimination follows Michaelis-Menten type kinetics. The data from the lower doses (or higher doses) alone may not render enough information to estimate both the maximal velocity (Vmax) and concentration for half-maximal velocity (k_m). The same argument applies when estimating the parameters of an Emax model.

Nonlinear mixed effects modeling probably is the most powerful technique for analyzing experimental and observational data due to several reasons. Mainly, the NM method does not share the drawbacks of the other methods discussed above. Both stages of the TS method are performed in one step, hence NM technique is also known as the one-stage method. One of the chief advantages of the NM method is its ability to conduct meta-analysis that is valuable in summarizing data across a drug development program. The primary disadvantage of this method is the requirement of sophisticated software that is not necessarily user-friendly for a wider application. Usually, special training is required to use the software packages and the learning resources are limited.

Model Qualification Methods

Model qualification is more popularly known as model "validation." The word "validation" implies a procedure of utmost robustness and may not be applicable to the usual PK/PD models that are found in the literature. Further, the fact that the true model and its parameters are not known makes the choice of the word "validation" even poorer. A contrasting example would be the validation of an analytical method, where "true" concentrations of the chemical entity are known for making a calibration standard. For wider acceptance, all models are required to be qualified and

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credible. Clear specification of the purpose for which the model is being developed is a prerequisite for any model building exercise.

Qualified Model/parameters. A model and its set of parameters are deemed "qualified" to perform particular task(s) if they satisfy prespecified criteria. Example: Application of posterior predictive check to a model and its parameters for use in Monte Carlo simulations [4, 5].

Credible Model/parameters. A model and its set of parameters are deemed "credible" [6] to perform particular task(s) if the conceptual foundation on which the model was proposed is satisfactory to a group of experts (subject matter-experts). Although there is no formal record of the existence of such models, to the best of our knowledge, we speculate that (at least the structural) models for warfarin [7] and reverse transcriptase/aspartyl protease inhibitors [8] would be deemed as "credible."

Monte Carlo simulations can be used to qualify a given model and its parameters. Based on the objective, qualification methods can test either the descriptive capacity or the extrapolation capacity of a given model. Adequate description of the data will ensure that the proposed model and its parameters are qualified to make inferences reliably within the range of the data studied. Such a qualification will be assessed using the routine diagnostic tests such as plots of the independent variable vs. observed and (individual/population) predicted, summary statistics and determining the precision of the parameter estimates. For example, developing an acceptable descriptive model is critical for making labeling recommendations. Product labels, usually, do not extrapolate results beyond the data range observed. A model is qualified to predict beyond the range of the data used for building the model if the descriptive capacity of the model is acceptable and the model (and parameters, if applicable) is credible. It is important to note that there is no means of assessing whether a model can be used for extrapolation. Hence the credibility of the model i.e., whether the model was derived from sound physiological principles and whether the submodel and its parameters appear reasonable to a panel of experts, is important.

The guidance for industry on population pharmacokinetics presents a variety of simulation methods that can be used to "qualify" models/parameters [7]. Although a variety of methods for model qualification are known, no thorough evaluation of their advantages and disadvantages is available.

Model Based Dosage Optimization

Upon the selection of the appropriate PK/PD model, optimal dosage needs to be derived for each patient. Two new "models" are introduced at this point—the cost and the utility functions. The cost-utility analysis in PK/PD

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modeling is relatively new and only the general theoretical principles will be discussed here. The cost of a therapy can be defined as the "expense" of the therapy due to an adverse effect, given the desired effect. Consider two drugs—one for relieving migraine headache and another for treating subarachanoid hemorrhage. Assume that both these drugs produce nausea. Given the indication (migraine versus stroke), the cost of the two therapies could be drastically different and hence may need different weighting. The physician(s) and/or the patient decide the "cost" of a therapy, which makes it highly subjective. The utility of a therapy can be defined as the advantage the therapy is providing over not taking the therapy, given the cost of declining therapy and the cost of drug-related toxicity.

$$\text{Utility} = f(\text{Cost (No Therapy)}, \text{Benefit}, \text{Cost (Toxicity)}) \quad (3)$$

The utility function could have many components depending on the number of desired and undesired effects. Figure 2 shows the concentration (or dose)effect curves and the utility curve for various costs. Using the curves such as those in Fig. 2, a target exposure and the region of therapeutic equivalence should be determined. For example, the curve in Panel B for the stroke drug suggests an optimal target exposure of about 100. Further, the utility equivalence region would be, say, between 80 and 500% (asymmetric

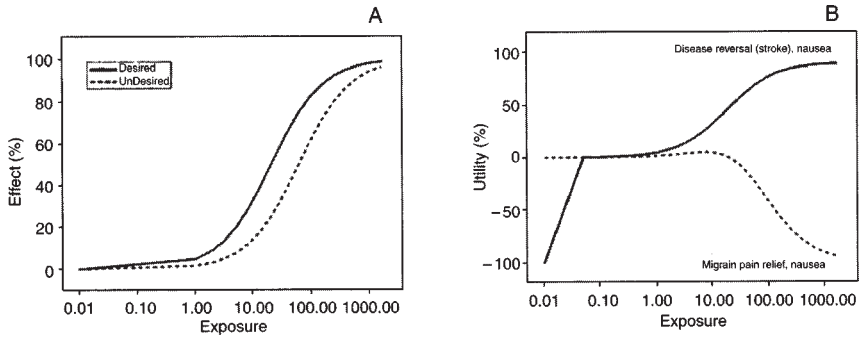

FIGURE 2 The exposure (concentration or dose)-response (desired and undesired) relationships of a hypothetical drug (Panel A). The utility of the therapy was determined by subtracting the (cost adjusted) undesired effect from the desired effect. The utilities of the therapy for two different desired effects [disease reversal (stroke), migraine pain relief] given the same undesired effect (nausea) are shown in Panel B. Note that declining therapy for stroke has a high cost. The exposure that results in the maximum utility would be the optimal target exposure. In Panel B, the optimal target exposure would be about 100 for the stroke drug and zero for the antimigraine drug.

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intervals). The corresponding exposures can then serve as surrogates for individualizing drug exposures and establishing equivalence of two products.

REGULATORY INITIATIVES

Several guidance documents for industry issued to date, reflect the leadership of FDA in improving current drug development and in embracing good scientific principles in the regulatory decision-making. Important messages to industry, extracted from few guidance documents, are highlighted here.

International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) E4 [9]

The guidance for industry on dose-response information to support drug registration states the use of a concentration-(desired/undesired) effect relationship in individualizing therapy, optimal dosing regimen, and for purposes of preparing dosing instructions in the product label. It further notes that knowledge of the dose-response relationship enables multiple regulatory agencies to make approval decisions from a common database.

Food and Drug Administration Modernization Act (FDAMA)

The implications of the FDAMA are discussed in the guidance for industry on providing clinical evidence of effectiveness for human drug and biological products [10]. Demonstrating effectiveness of a new drug product usually requires more than one adequate and well-controlled investigation. A full section entitled "extrapolation from existing studies" is devoted to presenting a nonexclusive list of scenarios when additional clinical studies are not necessary. The premise is that an acceptable benefit-risk ratio of a drug product has already been established. Controlled clinical trials are not necessary for approval of such a product for pediatric use and for establishing equivalence of alternative formulations, modified-release dosage forms, and different doses, regimens, or dosage forms. It is important to note that the guidance emphasizes the availability of well-defined concentration-effect relationships in the original new drug application. The sponsors can very effectively take advantage of this provision by prospective planning of the drug development programs.

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Pediatric Exclusivity

The FDA offers a six-month extension of the patent on the use of a new drug, should the sponsor fulfill the written request to characterize the PK/ PD of the drug in pediatrics. As discussed in the above section, additional adequate and well-controlled studies may not be required.

Recent Advisory Committee Meetings

The proceedings of two recent advisory committee meetings, one for the antiviral (AV) drug products and the other for the cardio-renal (CR) drug products, are noteworthy. Both these meetings devoted 50% of the total time to discuss the role of PK/PD in the AV and CR drug development. The AV advisory committee discussed the role of modeling and simulation in exploring various dosing regimens to appreciate resistance to the effects of protease inhibitors over cumulative exposure and the importance of compliance. The AV committee recommended that FDA should develop guidance to the industry on the role of PK/PD in developing AV drug products. The CR advisory meeting encompassed discussions on the need to determine the exposure-response relationship. The FDA presented retrospective dose-effect analyses of more than 10 antihypertensive agents previously approved over several years by the FDA [11]. The point that the dose-response range of most of the drugs did not allow adequate identification of the "optimum" dose was made. This affects several regulatory decisions such as approval of combination drug products and superiority claims. The outcomes of the meeting included: (1) use of modeldependent analysis to learn about the shape of the exposure-response curve and (2) need for more innovative designs that could potentially allow frequentist and Bayesian types of data analysis.

Guidance to Industry on Population Pharmacokinetics [12] and Exposure-Response

The guidance to industry on population pharmacokinetics emphasizes the role of modeling and simulation [13] in designing trials and analyzing trial outcomes. The exposure-response guidance focuses on the design and analysis of data from studies characterizing the PK/PD of a drug.

The impact of the aforementioned regulatory recommendations issued by the FDA is obvious. With efficient planning, sponsors can economize drug development time and resources, and take full advantage of the incentives. Building a concentration (not dose)-biomaker/surrogate/clinical endpoint relationship during the development of a new drug for use in adults can readily facilitate design (using simulations), analysis, and dosing

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recommendations (labeling changes) for the drug's use in pediatrics. However, the ability of a concentration-effect relationship to support approval of a dose/regimen not directly studied in clinical trials is not being fully exploited. This is in fact one of the strongest uses of modeling and simulation. Usually doses/regimens "directly" studied in clinical trials are proposed in the labels. A model can effectively be used to explore the suitability of intermediate doses not directly studied but could potentially offer similar effectiveness as the other doses or dosing regimens. Extrapolating outside the studied range may not be possible.

APPLICATIONS

Integration of Clinical Pharmacology Knowledge

The typical drug development strategies include dose ranging and bridging studies. The dose ranging studies can be employed to model the concentration (or dose)-effect (desired/undesired) relationships. The clinical pharmacology characterization of a new drug involves a variety of bridging studies to understand the influence of prognostic factors, such as age, gender, smoking habit, food, hepatic/renal impairment, etc. Effectiveness and safety data may not be collected in these types of studies, but could be simulated from the previously developed model. A recent example from a new drug application review is noteworthy. The dose-pain relief (desired effect) and the concentration-heart rate (undesired effect) relationship of a new drug, were both developed by meta-analysis of various clinical studies.

In other studies, patients with severe renal impairment demonstrated a 60% decrease in the systemic clearance compared to that in normal subjects. The influence of a 60% change in the drug exposure on effectiveness and safety was simulated. Dosing without any adjustments in renal-impaired patients causes negligible increase in the probability of pain relief and heart rate. There is 100% probability that the increase in heart rate is within three beats per minute. Whether a particular probability of occurrence of a given magnitude of change, in the effectiveness and safety of drugs, due to prognostic factors, is clinically relevant or not has to be mutually discussed with the clinicians (domain-experts). The M&S offer a powerful method to integrate knowledge across a submitted application. Simulating the probability distributions of effectiveness and safety for the bridging studies would enable a more informed and scientifically sound decision-making regarding the necessity for a regulatory concern. Preserving and accessing the knowledge when necessary at a later point of time will be much easier and efficient. Further, such simulations can be instrumental in the

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determination of exposure-equivalence intervals for the approval of changes in the future formulations.

Special Populations

One of the most widely sought out labeling changes in special populations is that for pediatrics. The application of M&S towards establishing in vivo characteristics as a way to making labeling changes is worth discussing further. The pediatric exclusivity policy is previously described (Sec. 2.3). If there is reasonable belief that the disease process is similar in adults and pediatrics and further an acceptable pharmacological effect marker is available, then studies in pediatrics measuring the concentration-pharmacological effect(s) can be potentially used to recommend dosing changes in pediatrics. The question that is being posed in the pediatric studies is: "Are the pharmacokinetics/pharmacodynamics in pediatrics predictable from those in adults?" Such a question can only be answered by developing concentration-effect relationships. The sponsors are encouraged to employ the model developed based on the PK/PD data in adults to design trials in pediatrics. The analysis of the PK/PD data from trials in pediatrics may require combining data from adults for a more complete understanding of the drug behavior.

Influence of Prognostic Factors

One of the aims of modeling is to identify influential prognostic factors such as body weight, age, gender, food, smoking habits, etc., on the fundamental PK/PD parameters. Nisoldipine is formulated as a once-a-day controlled release formulation of a dihydropyridine calcium channel antagonist which is approved in the United States for the treatment of hypertension. Food was found to increase the maximum concentration (Cmax: 2.75 vs. 7.5 μg/L) and decrease the extent of bioavailability (AUC: 70.4 vs. 53 μg.hr/L) of the controlled release product. The influence of the higher concentrations on the decrease of blood pressure was evaluated using a previously developed concentration-effect (blood pressure) model [14]. Simulations of the effect under the Fed condition allowed in alleviating the safety concern of a large drop in blood pressure. However in the labeling of Sular, administration on an empty stomach for optimal bioavailability was recommended. The docetaxel PK/PD relationship, in patients with cancer, was successful in identifying a subpopulation, patients with liver impairment, to be more prone to neutropenia (grade 4) [15]. This important finding was the basis for the dosing recommendations in the labeling, for patients with liver insufficiency. The drug development program of docetaxel exemplifies the

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value added by the incorporation of prospective planning on the use of M&S into the clinical trials.

FUTURE CONSIDERATIONS

M&S Team Structure/Communication

The biggest challenge, in the implementation of M&S projects, institutions face today is team structuring and communication. Successful execution of an M&S project undoubtedly requires teamwork and cooperation among scientists from various disciplines (e.g., clinical, pharmacometrics, statistics) and institutions (such as FDA and the industry). As aptly noted by Sheiner [16], a clear definition of the roles of the "domain experts" (such as clinicians/regulators) and "subject matter experts" (such as pharmacometricians/statisticians) is the key to success and efficient management of an M&S project. The domain experts would provide the answers for the questions: (1) What do we want to know? (2) What are we willing to assume, and (3) how certain do we need to be? Once the answers for these questions are provided the subject matter experts will provide the suitable experimental designs and analyses plans. It would take few iterations to arrive at the final answers (which are in fact questions) and a prospective design to achieve them. The M&S can be used as a very effective tool during these "iterations." Now, this exercise is particularly effective when the discussions are between the regulatory agency and a drug sponsor. The regulators will be in a position to comprehend "quantitatively," the rationale for the selection of a particular clinical trial design, in a timely fashion. Further, the pharmacometricians and statisticians, who are the designated "subject matter experts," need to have a more active exchange of knowledge across the two disciplines.

Pharmacometrics Training

The sources of learning pharmacometrics-related subject matter are very limited. This situation needs to be addressed immediately for widening the scope of M&S use. A pharmacometrician should have knowledge of basic PK/PD concepts, adequate statistics background, good understanding of physiological principles, and hands-on experience with at least one software which can be used for M&S and another one to conduct statistical analysis. Pharmacometricians also need to be trained in communicating "effectively" with clinicians and statisticians. Regulatory agencies play a vital role in emphasizing the importance of this discipline, as supported by the various regulatory initiatives, discussed earlier. Industry should, then, recognize the

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need for pharmacometricians and the academic institutions should train them. A long-term solution, then, would be for the academic institutions to offer graduate studies in pharmacometrics. A short-term solution is internal training. The pharmacometricians within the institutions should venture in collaborative projects thereby sharing the experience with the rest. Part of the problem is also the practice of M&S as an art rather than a science. Initiatives in streamlining the model-building process and making the simulation exercise more transparent and reproducible are critical.

Time Intensity

Model building takes a longer time than performing and analyzing simulations. Retrospective model building has two major steps—(1) data access and (2) data analysis. The former is probably the rate-limiting step. Typically, models are developed at the end of phase 3, most of the times. A prudent way to economize time to develop models is by incorporating what can be called as a "progressive model building (PMB) paradigm." The essence of the PMB paradigm is to update a model as new knowledge is accrued. The PMB is advantageous because of at least two reasons. The first one is being able to "carry-forward" the knowledge all along the drug development for a given product and the second one is being able to divide a big problem into several small components ("divide and conquer") that are easier to achieve. However, implementation of this paradigm calls for more open collaboration of scientists from all disciplines and institutional commitment to use the "current" model in designing the next trial. By utilizing the PMB paradigm, scientists are almost forced to employ mechanistic models, since the generalization power of empirical models is limited. For example, it is much easier to update the parameter estimates of an Emax model (with covariate effects) from a latest trial compared to those of a cubic-spline model.

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Scientific and Regulatory Considerations for Studies in Special Populations

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INTRODUCTION

The course of development of an individual organism through successive transformations in a lifetime is referred to as ontogeny. Consequences of developmental changes and thus drug dosage modifications based on age, liver function, renal function, and other intrinsic and extrinsic factors have been well known for some time. Some examples of intrinsic factors are genotype, gender, ethnicity, inherited diseases, acquired diseases, age specific diseases, and polymorphism, and examples of extrinsic factors include smoking, drug abuse, environmental pollutants, xenobiotic exposure, and diet factors. During drug development it is not always possible to include enough number of patients in pivotal clinical trials, to represent each subpopulation. These subpopulations—also called special or specific populations—include different ethnic and racial groups, age groups, genders, pregnancy, lactation, and certain types of disease states (liver and renal impairment) which may affect drug disposition, obesity, smokers, etc. Pharmacokinetic (PK) and/or pharmacodynamic (PD) differences for all

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these subgroups have been reported in the literature. In this book, pregnancy and lactation have been discussed in Chapter 13, drug-drug interaction in Chapter 14 and effects of certain disease states have been presented in Chapter 15.

This chapter will introduce the readers to:

1. Some of the PK and/or PD differences reported for race, age, gender, and obesity.
2. Regulatory perspective for gender, race, pediatric, and elderly populations.
3. Study design considerations commonly used to assess differences in specific populations.
4. Dose adjustment strategies.

As one can appreciate, this chapter is just an introduction to assessing differences in important demographic subgroups and regulatory perspective, it is not an extensive review and in no way a comprehensive discussion of this vast field of special populations. Readers should also refer to Chapter 2 of this book for regulations on special populations. The main discussion in the following paragraphs will only focus on gender, race, elderly and pediatric populations.

One of the major roles of clinical pharmacology is to provide information which will aid in the individualization of the dose and dosing regimen. As discussed later on, to identify when dosage adjustment may be necessary, it is important to identify the limits of change in exposure of the drug that can be accepted/tolerated for the drug being developed [1]. Once we have identified the change in exposure that can be tolerated, one can recommend adjusting the dose if that threshold has been reached in a specific population, or in cases of drug-drug or drug-food interactions. Dose adjustment strategies have been discussed later in the chapter.

DATA SUPPORTING THE NEED TO ASSESS DIFFERENCES IN SPECIFIC POPULATIONS

Gender

Several examples have been reported in the literature that shows gender-dependent pharmacokinetic and pharmacodynamic differences [2–21]. The investigators have reported that the many differences in ADME based on gender cannot be explained by differences in body weight or body composition.

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Absorption of most drugs is a passive process and depends on factors such as PKa, lipophilicity, and gastrointestinal physiology. Women secrete less gastric acid and have slower gastric emptying than men. The mechanism of this is unknown but has been hypothesized to be related to differences in steroid hormone levels due to exogenous hormones and pregnancy [22, 23]. Gender specific absorption is rare and known examples are not found to be clinically relevant [24, 25].

Distribution of drugs is influenced by physico-chemical properties, vascular and tissue distribution, and ratio of lean body mass to adipose tissue mass. Gender differences in drug distribution are related to body weight and/or body fat proportion, whereas, plasma protein binding differences are minor, and not of clinical significance [8, 19]. Many gender differences are attributed to significant gender specific differences in drug metabolism [15–17]. Total clearance of several CYP3A substrates appears to be faster in women compared to men. Drugs metabolized by cytochrome CYP1A, CYP2D6, CYP2E1, and Phase II metabolism such as glucuronidation, conjugation, glucuronyltransferases, methyltransferases, dehydrogenases, and by combined oxidative and conjugation processes are usually cleared faster in men compared to women. Drugs metabolized by CYP2C9, CYP2C19, and *N*-acetyltransferase, appear to have no gender effect [3, 20]. Glomerular filtration, tubular secretion, and tubular reabsorption appear to be faster in men compared to women [20]. Thus there are varying degrees of gender-dependent clearance for several drugs. Some drugs are cleared faster in females than in males, while some are cleared faster in males than in females, whereas, many drugs have no gender-dependent differences in their pharmacokinetics. Moreover, because of the difference in maturation of each gender (for example, age at which puberty is reached), many genderdependent pharmacokinetic characteristics of a drug may be manifested as age-dependent factors [8].

The inclusion of women in clinical trials, and assessing gender differences for the data obtained from pivotal clinical trials has been emphasized by the FDA since two decades [27]. The Institute of Medicine has defined gender difference as a difference between men and women due to cultural or social variations in a particular sex. A sex difference has been defined as a difference due to the sex chromosome or sex hormone [20]. The FDA has described cultural, social, genetic, or hormonal differences between males and females and used the term “gender differences” [2, 20, 21].

Literature has several excellent reviews summarizing the gender specific differences in ADME and Pharmacodynamic variables. In general, pharmacokinetic variability in gender has been better characterized compared to pharmacodynamics variability. Limitations in measurements of pharmacodynamic effects pose limitations (e.g., difficulty in quantifying depression or perception of pain) [3]. Despite these limitations several

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gender specific response data have been published [3, 4, 8]. Some of the examples reported for pharmacodynamic differences include, women having a better response to monoamine oxidase inhibitors (MAO) than to tricyclics; more sensitivity to effects of ethanol; greater magnitude of response to SSRIs, and; greater adverse events to cardiovascular drugs [8, 9, 20].

Race

The majority of literature information on PK and/or PD differences for race is comparisons between Caucasians and Asians (often Chinese), and African Americans and Caucasians. The influence of ethnicity on ADME characteristics and PD of drugs have been reported and reviewed extensively in the literature [29–39].

Drugs undergoing passive absorption are not expected to have any differences. Calcium absorption is an active process and the fraction absorbed in Caucasians is 25% vs. 44% in African Americans [29]. This suggests that drugs undergoing active absorption may exhibit racial differences.

Ethnic specificity in molecular genetics is one of the factors contributing to the interethnic differences in drug disposition and response. The human drug-metabolizing enzymes including CYP2D6, CYP2C9, CYP2C19, CYP2E1, CYP2A6, aldehyde dehydrogenase (ALDH2), alcohol dehydrogenase (ADH3) and non-P450 monooxigenase, *N*-acetyltransferase (NAT2), glutathione *S*-transferase (GST), catechol-*0*-methyltransferase (COMT), UDP-gucuronosyl-transferase (UGT), thiopurine methyltransferase (TPMT), and dihdropyrimidine dehydrogenase (DPD), all display polymorphism. Among these polymorphic enzymes, many of them had exhibited known ethnic specificity including CYP2D6, CYP2C9, CYP2C19, CYP2A6, UGT, NAT2, and ADH3 [40]. Further, gut metabolism via CYP3A4 or PGP transport may affect absolute bioavailablity.

A review of 339 literature citations by Bjornson et al. [39] concluded that no citation clearly described differences in active absorption of drugs involving P-glycoprotein (PGP) transporters, α-1 Acid glycoprotein (AAG) concentrations are reported to be lower in blacks and Chinese as compared to Caucasians whereas, amounts of albumin are similar in these three groups. Thus drugs binding exclusively to albumin are unlikely to show any racial differences whereas, drugs binding to AAG are likely to have higher binding, that is, a lower free fraction in Caucasians than in Chinese and African Americans. However, none of the reported differences are clinically relevant [39]. It may be advisable to assess race-dependent protein binding especially for drugs predominantly bound to AAG. There is a potential for

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race-dependent variability related to transporter [39]. Race-dependent differences in metabolism are extensively reported. The incidence of poor metabolizers of debrisoquinine phenotype in different populations for CYP2D6 is, 7% for U.S. Caucasians, 0.7% for Chinese, and 0.5% for Japanese, whereas, for CYP2C19 the incidence of poor metabolizers of mephenytion is 3% for Caucasians, 17% for Chinese, and 22% for Japanese. There are significant ethnic differences in enzyme activity of CYP2C9, 2C19, 2D6, 1A2, 2A6, and *N*-acetyl transferase [39]. Based on in vitro human liver microsomes of Caucasians vs. Japanese, 1A2, 2A6, 2D6, 2E1, and 3A4 enzyme activities are higher in Caucasians. Racial differences in acetylators have been recognized since a long time. The frequency of slow acetylators is as follows: African Americans 42–51%, Caucasians 52–58%, Chinese 22%, Eskimos (Canada) 10%, and Japanese 7–12% [29]. Fifty percent of Chinese and Japanese populations lack aldehyde dehydrogenase enzyme activity resulting in accumulation of acetaldehyde which could result in side effects like tacheycardia, palpitation, and facial flushing. In summary, hepatic metabolism differences are the most common ethnic differences.

Glomerular filtration and reabsorption being passive processes of excretion are not likely to be affected by race. Tubular secretion is an active process. In Chinese the renal clearance of metabolites of morphine is significantly higher, suggesting that tubular secretion may be affected by race [39].

The evaluation of drug response for several ethnic differences has also been recently reviewed [39]. Some of the reported differences include—African Americans having higher incidence of hypertension, interethnic differences in vasodilatory response, and Chinese patients requiring a lower daily dose of Warfarin. For drugs undergoing acetylation, populations with a greater number of slow acetylators are likely to experience greater number of adverse events. In addition to issues related to ethnic differences, other factors such as diet, socio-economic status, exposure to environmental pollutants, or interaction between these factors could play a role contributing to ethnic differences, especially for the populations living in the different regions of the world [41–48]. The effect of diet is not discussed in this chapter, but has recently been reviewed in the literature [49].

Elderly

Elderly is defined as 65 years of age or older. Physiological changes occur in aging which affect the ADME of drugs. The influence of age on pharmacokinetics and pharmacodynamics has been extensively reviewed in the literature [50–55]. In the elderly, the gastric pH is elevated, gastric emptying time slightly reduced; intestinal motility, muscular blood flow,

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plasma protein, and total body water are reduced; whereas, serum fatty acids and adipose tissue are increased [50]. Kinirons and Crome [50] have summarized the following accepted principles for elderly population: decline in renal function with age, significant decline in liver size and mass, significant reduction in hepatic blood flow; decreased cardiac output, metabolic and renal clearance; in vitro content and activity of CYP450 enzymes or conjugation enzymes are not reduced with age. However, in vivo clearance of drugs metabolized by CYP3A4, 2C9, 2C19, and 1A2 have been reported to be reduced whereas, no reduction in clearance of drugs metabolized by CYP2D6 and Phase II enzymes has been reported. With regard to renal function, GFR, tubular secretion, and reabsorption are all reported to be reduced in the elderly population. Differences in sensitivity to drugs have also been reported with age for CNS and cardiovascular drugs [50, 52].

Pediatrics

Children may exhibit different drug disposition and/or response compared to adults. The pediatric patient cannot be considered as a "little" adult. It is well documented that age-related developmental and physiological changes exist not only in the pediatric population compared to adults but also within pediatric age group. In addition, environmental (e.g., exposure to drugs in vitro) and dietary factors can affect PK of drugs [56]. FDA guidance on pediatrics and ICH E11 [57] define age groups within pediatric population. The pediatric population is categorized into the following age groups—preterm new born (gestation 23 to 34 weeks), term newborn infants (0 to 1 month), toddlers (1 to 24 months), children (2–11 years), and adolescents (12–16/18 years).

Absorption of drugs can be affected by gastric pH, gastric emptying time, and intestinal transit times. Gastric pH value is almost neutral at birth [6, 7] then starts to vary from day eight and slowly declines to reach the adult value by age three to seven years. This results in higher absorption of acid labile drugs, such as penicillin and amoxicillin in toddlers and younger children. Gastric emptying is prolonged until six months of age. Intestinal transit time is decreased in children resulting in incomplete absorption of sustained release products [58–61].

The total body water is increased and the percentage of body fat is decreased in infants and children [62]. Albumin concentrations normalize at one year of age and albumin binding is lower in infants. The concentration of AAG is also higher over the first year [63, 64]. The variability with age in these factors can affect drug binding and thus the drug distribution [65, 66]. Further, the blood brain barrier in newborn

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infants is not fully developed and drugs may cross the blood brain barrier resulting in CNS toxicity [56, 67].

Both Phases I and II metabolizing enzymes are not mature at the time of birth and different enzyme activity may reach the adult levels at different ages (Table 1). For example, CYP3A4 activity may reach the adult level at six months of age, whereas, CYP2D6 maturation occurs by five years and CYP1A6 by 10 years of age. In case of renal excretion, the GFR, active tubular secretion, and tubular reabsorption are lower in infants and nearly equal to adults by 12 months of age and reach adult levels by childhood.

P-glycoprotein (PGP) expression has been associated with decreased gut absorption of drugs and decreased amount of drugs crossing the blood brain barrier. However, developmental aspects of PGP have not been investigated [56]. Pharmacodynamic changes with age have been known for neuromuscular blocking agents [68, 69].

Obesity

Recent reports indicate that obesity in the United States and worldwide is on the rise [70]. Body mass index (BMI) is used to define obesity. Body mass index is the ratio of the weight in kilograms to the square of the height in meters [71]. The prevalence of childhood obesity has doubled in the last two decades [72]. Estimates suggest that about 16% of children in the United States may be obese. These estimates are higher in some minorities. Blounin and Waren define obesity as a disease state characterized as a condition from excess accumulation of body fat. Obesity is associated with changes in plasma protein binding constituents and increase in adipose tissue mass and lean body mass, organ mass, cardiac output, and splanchnic blood flow relative to normal weight individuals [73].

Absorption in obesity is poorly understood, overall no significant absorption differences in the obese compared to lean subjects have been reported. For obese patients, drugs with less lipophilicity have little or no change in VD. Increasingly lipophilic substances are affected by obesity. Drugs predominantly bound to albumin do not show any significant difference in protein binding [74–76]. AGP concentrations maybe higher in obese patients resulting in decreased free fractions [77].

The effect of obesity on metabolism has not been well studied. The activity of C4P3A4 is lower and that of CYP2E1 is higher in obese compared to nonobese [78]. The effect of obesity on cytochrome P450 1A2, 2C9, 2C19, and 2D6 is inconclusive. Glucoronidation is significantly increased and Sulfation may be moderately increased in obese [79, 80]. For excretion, GFR has been shown to increase [81, 82] in some citations, whereas it has also been shown to decrease [83]. This discrepancy has been

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hypothesized to be due to different degrees of obesity in different studies. Tubular secretion is possibly increased and tubular reabsorption is decreased in obese [80, 84, 85].

Georgiadis et al. [86] assessed toxicity of several chemotherapeutic agents to obese and compared toxicity to nonobese patients and concluded that there was no correlation between toxicity and obesity. Each drug behaved differently so predication of toxicity based on obesity was difficult. Therefore, careful monitoring of narrow therapeutic index has been recommended. When the same dose of triazolam [87, 88] was administered, obese patients showed increased sensitivity. Desensitization of acetylcholine receptors has been observed in obese [87].

With the incidence of obesity on the rise, it may become increasingly important to assess obesity as a covariate during drug development.

REGULATORY PERSPECTIVE

Gender

In 1977, FDA issued a guidance which recommended that all women of child bearing potential be excluded from clinical trials, unless adequate safety, efficacy, animal fertility, and teratology information was available for the drug being investigated [89]. This was done to protect the fetus, and the assumption that men and women metabolize and respond to drugs in a similar way [2]. In 1988, guidelines for the "format and content of the clinical and statistical sections of the drug application" were issued which required of the sponsors to discern dose-response relationships in the AEs and examination of rates of AEs in various demographics (age, race, gender) and other subgroups (metabolic status, renal function) [27]. In 1993, FDA revoked the 1977 guidelines and issued a guidance calling for the inclusion of analyses of efficacy and safety data by gender, and inclusion of characterization of pharmacokinetics of drugs in men and women. The "Refuse to file" (RTF) guidance published by the FDA also in 1993, stated that NDA could be RTF if there was "clearly inadequate evaluation for safety and/or effectiveness in the population intended to use the drug, including pertinent subsets, such as gender, age, and racial subsets" [90].

The U.S. FDA and other regulatory agencies have emphasized the need to include subgroups such as gender, age, and race in the clinical trials. In order to encourage recruitment of subgroups in clinical trails in all phases of drug development, the Demographic Rule [91] was published in 1988, which includes the following publications (2): for NDAs (21 CFR 314.50 (d)(5)(v) and (d)(5)(vi)(a)); and for INDs (21 CFR 312.33 (a)(2)) and the clinical hold

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rule. Guidance on Bioavailability and Bioequivalence issued by the FDA in 2000 also recommends that attempts should be made to include both sexes, and representative ages and race. The International Harmonization Conference (ICH) issued guidelines on clinical study reports (ICH E3) [92] asking to include demographics and subgroup information to evaluate safety and effectiveness in the subpopulations. It is evident that regulatory agencies including ICH require inclusion of subgroups such as gender, age, and race. The regulatory guidelines call for including enough number of subjects to perform subgroup analysis.

Labeling for Gender

Summary of the 330 NDA reviews of drugs submitted between 1994 and 2000 [93] revealed that 163 drugs had gender specific information of which 122 drugs were new molecular entities (NME) and 39 of these drugs had gender specific pharmacodynamics data. Eleven of these drugs were identified as having greater than 40% differences in PK parameters. These differences were described in the clinical pharmacology, special population, or in the precaution sections of the drug labeling. Eight of the 39 drugs with gender-related pharmacodynamic information, reported gender based PD differences. Five reported increase in adverse events in females (neutropenia, thrombocytopenia, QTC changes, risk of Torsade de Pointes, and other mild adverse events), three drugs reported higher response in females compared to males. These PD differences were not necessarily related to PK differences. Of the eight drugs reporting differences in PD, five had less than 20% difference in PK parameters.

Toigo et al. [94] evaluated clinical review of the drugs approved between 1995–1999 to assess the participation of women in clinical trials and gender-related labeling. Based on the review of clinical trial protocols and labeling of 185 NMEs, they concluded that the participation of women in clinical trials was proportionate to their representation in the U.S. population. Labeling of 66% of drug products contained statements about gender; only 22% described the actual gender effects. About 90% of the gender effects discussed was PK related, 12% safety related and 5% efficacy related. None of the labels recommended dosage adjustment for women.

Race

In 1985, the first regulation on special populations, 21CFR 314.5 asked for evidence to support the dosage and administration section of label for specific populations. In 1993, NIH published guidelines and they have been updated in 2001 [95], which directed that appropriate proportions of women and minorities be included in NIH sponsored clinical research.

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These NIH guidelines called for review of the data to show whether clinically important gender and minority based differences are expected. If differences in response are expected then the phase III trial should be designed to answer questions and include adequate sample size for subgroups. The 1998 Demographic rule on IND and NDA requires that Sponsors include analysis of effectiveness and safety, and modification of dose and dosage regimen, for important demographic subgroups including race (21 CFR 314.50 (d) (5) (VI) (a)). As stated in the section for gender above, 21 CFR 314.10 (d) (3), FDA may refuse to file an NDA if pertinent analysis for subsets of population is not included in the application. International conference of Harmonization E5 (also printed at 63FR 31790, June 1999) documents issued in 1998 describe the importance of evaluating impact of ethnic factors on drug's safety and efficacy. Since the ICH format will allow the same application to be submitted in different regions of the world it is important to evaluate the impact of ethnic factors, for acceptability of data generated in foreign countries/populations. One of the major issues in extrapolating clinical data from one region to another region is the potential impact of ethnicity on the drug's pharmacokinetics, pharmacodynamics, drug efficacy, and toxicity [32].

To ensure consistency in subset analysis across studies, and to ensure potential subgroup differences in a meaningful way, FDA is now recommending [96] use of the standardized Office of Management and Budget (OMB) race and ethnicity categories. This guidance recommends that race and ethnicity information be a two-question approach and subjects in a study self report that information. For ethnicity, two minimum choices be offered, Hispanic or Latino, and Non-Hispanic or Latino. For race the choices that be offered are American Indian or Alaska native, Asian, Black, African American, Native Hawaiian or other Pacific Islanders, and White. More detailed race and ethnicity information may be described but the characteristics should be traceable to the five minimum categories described above. Further, if studies are conducted outside the United States, the race and ethnicity categories suggested in the guidance may not be adequate to describe racial and ethnic populations in foreign countries. Therefore, it is important that the information collected in foreign populations be traceable to the recommended categories. The categories recommended are the same as for U.S. population, with the exception that the black or African American category can be replaced with a black or African heritage category.

There have been several regulations recommending that the sponsor include subgroup populations in the clinical development program. For example, the Population PK guidance [97], Exposure-Response Guidance [1], Content and Format of adverse reaction section of labeling for human prescription drugs and biologies [98], clinical section of labeling [99], and

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Best Pharmaceutical for Children Act, all ask for monitoring the race and ethnicity of children participating in clinical studies. It is evident from the regulations that are currently in place that regulatory agencies require adequate participation and evaluation of racial and ethnic differences in drug response.

Labeling for Race

Toigo et al. [100] reviewed 185 NMEs (approved for 1995 to 1999) for participation of racial and ethnic subgroups in clinical studies. The review findings were based on 2581 clinical trial protocols. They reported that 53% of clinical trial protocols had identified race. Whites represented 88%, Blacks 8%, Asians pacific islanders 1%, and Hispanic Latinos 3%. For Blacks the participation was consistent with the representation in the U.S. population, while Hispanics appeared to be lower than their representation in the U.S. population.

Review of these 185 drug labels [100] revealed that 84 (45%) had race related statements. Fifteen of these labels contained 18 statements indicating differences (9/18, 7/18, and 2/18, for PK, efficacy, safety related, respectively) due to race/ethnicity. Ten, one and five product labels were related to Blacks, Hispanics, and Asians, respectively. One antihypertensive drug label recommended higher doses in Blacks based on racial differences.

Elderly

As discussed above, ADME and pharmacodynamic response may be affected with increase in age. To prevent or reduce the risk of adverse events in the elderly, regulatory agencies have asked that the sponsors of new drugs include sufficient number of elderly (65–75 years) and very elderly (greater than 85 years of age) subjects in clinical trials. In 1977, the FDA established the geriatric use subsection for labeling [101] to include information for the elderly (21 CFR 201.57 (f) (10)) in the precaution section of the label. This labeling regulation requires that all marketed drugs submit revised labeling to include geriatric-use information. For details of this regulation refer to the FDA website for relevant guidances. As stated earlier, the "Format and content regulations" (63 FR 6854) require safety and efficacy data for important demographic subgroups including age be included. IND regulations (21 CFR 312.33 (a) (2)) require that annual reports by the sponsors should contain the information on number of subjects enrolled in clinical trials for certain subgroups including age. The "Content and forma for geriatric labeling" guidance has been published in October 2001 and

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gives a detailed procedure for submitting the "Geriatric Labeling Supplement". ICH guidelines also recommend inclusion and analysis of data for elderly—ICH-E7, "studies in support of Special Population—Geriatric"

Labeling for Elderly

To assess the availability of data on geriatric use in the label Sahajwalla and Kwon (unpublished data) conducted a survey of 2002 Physicians Desk Reference (PDR). A list of drugs was obtained by searching the key word "elderly" in the electronic version of the PDR. Six hundred and fifty two drug labels were listed with the key word "elderly," eliminating different dosage forms of the same drugs reduced this to a total of 549 drugs with elderly information. The clinical pharmacology, precaution, and dosage administration sections of these labels were reviewed. Out of 549 drugs, 141 drugs required dosage adjustments, 283 recommended cautions without recommending a dosage adjustment, 103 did not require any dose adjustments, and 22 drugs did not provide specific recommendation. Of the 141 drugs recommending dosage adjustment, 28 were based on PK findings, 100 due to PD findings, and 13 due to PK/PD findings. Forty one drugs recommended decrease in the dose by 30 to 50%, and 10 drugs recommended reducing the dose by more than 50%. Increased dosing interval was suggested for four drugs and 82 drugs did not specify how much dose reduction, but starting at a lower dose was recommended. Caution for 263 drugs was advised in the label due to PK changes, increased sensitivity, increased side effects, or the expected decreased renal, hepatic and cardiac function in elderly. It is clear from these findings that during drug development evaluating the effect of age on PK and PD of drugs is essential.

Pediatric

The need for inclusion of pediatric information in the drug label has been recognized by many drug regulatory agencies in the world. To encourage pediatric labeling a final pediatric rule was issued by the FDA in 1994 [102], which allowed adult efficacy data to be applied to pediatric patients with the same disease or condition by supplementing and supporting the indication with dosing and safety data in pediatric populations. In 1996, the content and format for pediatric use supplement was issued [103]. In 1997, the Food and Drug Modernization Act (FADMA) offered an incentive of six months extension of exclusivity to market the drug product if studies were performed in response to the FDA written request for pediatric studies. Readers can refer to FDA guidelines on qualifying for Pediatric Exclusivity

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under section 505(A) which was issued on June 30, 1998. In December 2001 FADMA expired, and in January 2002 the Best Pharmaceuticals for Children Act went into effect, which provided similar incentives as the FADMA. Other drug regulatory agencies in the world have also issued guidelines to conduct studies in pediatric populations and to include these populations in the product labeling. In August 1997, the Therapeutic Products Directorate, Canada issued the "Inclusion of Pediatric Subjects in Clinical Trials" guideline: in October 1997, the Australian Drug Evaluation Committee issued a report of a working party on the registration of drugs for use in children. In July 2000, ICH issued E 11 'Clinical Investigations of Medicinal Products in Pediatric Population.'

In order to decide if only PK study with safety data is sufficient to support pediatric indication or conduct of a PK and safety/efficacy trial will be needed, a decision tree has been published in the FDA's exposureresponse guidance and presented below as Fig. 1.

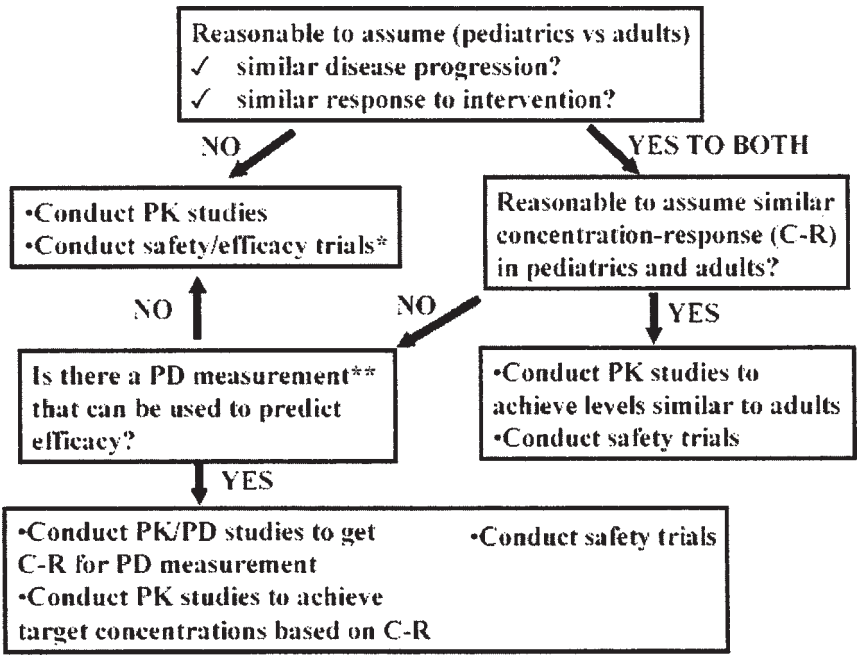
Reasonable to assume (pediatrics vs adults)
✓ similar disease progression?
✓ similar response to intervention?
NO
YES TO BOTH
•Conduct PK studies
•Conduct safety/efficacy trials*
Reasonable to assume similar concentration-response (C-R) in pediatrics and adults?
NO
NO
YES
Is there a PD measurement** that can be used to predict efficacy?
•Conduct PK studies to achieve levels similar to adults
•Conduct safety trials
YES
•Conduct PK/PD studies to get C-R for PD measurement
•Conduct PK studies to achieve target concentrations based on C-R
•Conduct safety trials

FIGURE 1 Pediatric study decision tree.

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STUDY DESIGN CONSIDERATIONS FOR SPECIAL POPULATIONS

The goal of clinical pharmacology studies in special populations is to determine how the dose and dosage regimen should be adjusted in special populations so that the same systemic exposure that was found to be safe and effective in the pivotal clinical trial for the population it was tested in can be achieved. There are two approaches that can be adopted, a standard PK approach and a population PK proach.

In a standard PK Approach, a single dose or multiple dose(s) of the drug are administered (within the same study protocol) to the population being investigated, e.g., males and females; different ethnic and race groups, adult vs. elderly, and diffent age categories in the pediatric age groups. The number of subjects included should be enough to obtain a reasonable estimate of variability. Following the administration of the drug, frequent blood and urine samples are collected and pharmacokinetic parameters estimated and compared between the various populations of interest.

With the population PK (POPPK) approach, fewer samples are collected from a larger number of subjects as compared to the Standard PK approach, and PK parameters obtained are compared between the populations of interest. The conduct of Population Studies is described in Chapter 11 and in the FDA Guidance on Population PK [97]. Population PK studies are generally conducted as an add-on study to Phase II and III clinical trials. Some of the advantages of this approach include fewer bloodsample collections. Thus ethical concerns of collecting several blood samples from certain populations (e.g., pediatric) are reduced. The sample collections can be part of a routine clinical visit when blood and urine are being collected for other laboratory investigations. Since these studies are generally being conducted as part of Phase II and III trials, phramacodynamic endpoints can also be measured and exposure-response (safety and efficacy parameters) relationships could be evaluated in different populations of interest.

In order to decide which approach (standard PK vs. Population PK) is better suited for conducting studies in special populations one should consider the following. Regulatory agencies worldwide require the inclusion of representative special populations in clinical trials, thus special populations will be part of Phase II and III clinical trials. Therefore data which can provide exposure-response (safety and efficacy parameters) measures by including POPPK in the special population within pivotal clinical trials would be more valuable than simply collecting information on pharmacokinetic differences based on the standard PK approach. Based on simulation studies some researchers believe that the population PK approach is preferred over the traditional PK approach when characterizing

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PK and PK/PD differences involving intrinsic (gender, race, age) factors. For assessing the effect of extrinsic factors (different drugs, smoking, food, etc.) one may not have enough subjects with the presence of that factor, enrolled in clinical trails to assess differences based on POPPK.

DOSE ADJUSTMENTS

An important factor in deciding the dose adjustment is the knowledge of exposure-response relationship [1]. Delineation of no-effect boundaries, based on dose- and/or concentration-response studies would be beneficial. Once the influence of intrinsic and extrinsic factors on drug exposure has been characterized and exposure-response has been established, appropriate dose adjustments can be recommended. Guidance on special populations (hepatic, renal) and extrinsic factors (food effect, drug interactions) recommend that in the absence of exposure-response data, the employment of a standard 90% confidence interval of 80–125% for AUC and C_{max} can be used. If differences for populations of interest are within these boundaries then dose adjustments are not needed. These guidances also acknowledge that "FDA recognizes that documentation that a PK parameter remains within an 80–125% *no effect* boundary would be very difficult given the small numbers of subjects usually entered into these studies. If a wider boundary can be supported clinically, however, it may be possible to conclude that there is no need for dose adjustment."

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Conducting Clinical Pharmacology Studies in Pregnant and Lactating Women

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INTRODUCTION

Pregnant and lactating women are two special populations that present unique challenges for conducting research. Many women of reproductive age group (15–5 year) may have chronic medical problems and use a variety of pharmaceutical products (e.g., drugs, vaccines, and other biologic therapies). In the U.S., 60 million women are of reproductive age (15–44) [1], and there are about four million births per year [2]. The magnitude of major chronic conditions in women less than 45 years is significant. In this population, asthma affects 6,099,000 women; epilepsy affects 466,000; and hypertension affects 2,700,000 [2]. The prevalence of these conditions among pregnant women are 7% for asthma, 0.6–1.0% for epilepsy, and 6% for hypertension [2]. Thus, many women enter pregnancy with medical conditions that require ongoing or episodic treatment. New medical problems may develop or old ones may be exacerbated by pregnancy (e.g., infections, migraine headaches, depression). Lactating women, as well, may require medication for chronic or acute conditions.

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Pregnant and lactating women are usually not part of the traditional drug development program. As a matter of fact, pregnant and lactating women are actively excluded from most clinical studies. If pregnancy does occur during a clinical study, treatment is discontinued and the patient is frequently dropped from the study. Consequently, at the time of initial marketing, except for products developed to treat conditions specific to pregnancy (e.g., tocolytic agents for preterm labor, treatment of preeclampsia), there are usually no data on the appropriate dosage and frequency of administration during pregnancy. The same situation may also be seen after years of marketing; data in product labels regarding pharmacokinetics and dose adjustments during pregnancy and lactation rarely provide more information than was available at the time of initial marketing.

Decisions can and should be made during drug development to study the kinetics of products in these subpopulations. If the drug is anticipated to be used by women of reproductive age, then developers should consider when and how to study pregnant and lactating women because the drug will be used by them once marketed. If a drug has a good maternal- and fetalsafety profile, studies can be performed in pregnancy. Pharmacokinetic/pharmacodynamic (PK/PD) studies in pregnant and lactating women should be considered if the drug is prescribed in or used by pregnant and lactating women or pregnancy or lactation are likely to significantly alter the PK of a drug (e.g., effect of pregnancy on a drug that is renally eliminated). These studies are especially important if use of the drug would be required and not optional to treat maternal medical conditions. If there is no systemic exposure to the product, or the product is not used by women of childbearing age, during pregnancy, and lactation there may be no need to conduct PK/PD studies.

The medical literature provides information about drugs being used in pregnant and lactating women and should help investigators select products for further study. Information on human pregnancy and lactation exposures and experiences usually emerge during the postmarketing phase for pharmaceutical products. Postmarketing data that demonstrate fetal and maternal safety help reduce the obstacles to performing PK studies in pregnancy. Publications in the medical or lay press may describe use of a drug in pregnancy and medical specialty groups may publish position statements or clinical recommendations for specific drug therapy for clinical scenarios. Publications may describe safety or efficacy in lactating women, safety in the breast-fed child via exposure to drug in breast milk, case reports describing use of a drug in lactating women, and information from medical specialty groups (e.g., consensus documents or opinion papers). These sources can help with determining the research questions to be investigated, and will additionally be useful when designing a protocol and informed consent documents, and obtaining IRB approval.

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Health care providers and their patients must make decisions about the use of medications during pregnancy and lactation with little to no data to guide them in decision-making. The ultimate goal of PK/PD studies in pregnant and lactating women should be to provide meaningful information for patients and their health care providers so that they can make informed decisions about drug use and appropriate dosing during pregnancy and lactation. Studying Pharmaceuticals in pregnancy and lactation requires special considerations including methodological design, data analysis, and ethical and regulatory considerations. When studies are performed in pregnant and lactating women, frequently the study utilizes only a few women. In addition, methodologies are often inadequate to draw substantial conclusions and have little influence on clinical prescribing scenarios.

This chapter will address considerations for investigators who recognize the importance of drug use in pregnant and lactating women, the need for data to assist prescribing, and despite the obstacles, choose to study pregnant and lactating women.

PREGNANCY

Introduction

Although the ideal situation during pregnancy is abstinence from the use of pharmacologic agents, many women use prescription or over-the-counter drugs during pregnancy. Several studies have shown that pregnant women do use prescribed or over-the-counter drugs during pregnancy [3–5]. A survey of approximately 20,000 women over a 25-year period (1976–2000) demonstrated that drug (excluding vitamins and minerals) use in pregnancy is increasing [6]. The mean number of drugs women reported using during pregnancy over this 25-year period has increased from 1.7 to 2.9. Over 80% of all women reported using any drug during pregnancy, and approximately 30% reported using>four drugs. In addition, of the top 10 reported drugs used, six were over-the-counter (OTC) products. In Europe a comparison of therapeutic drug use during pregnancy showed that 64% of women used at least one drug during pregnancy [4]. In France, pregnant women were prescribed an average of five drugs during the first trimester [5].

Physiology of Pregnancy

Pregnancy is a dynamic state of altered physiology. The physiologic changes inherent to pregnancy can affect the pharmacokinetics and/or pharmacodynamics of drugs (Table 1).

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TABLE 1 Physiologic Changes in Pregnancy with Potential to Alter ADME

Absorption	Delayed gastric emptying
	Prolonged gastrointestinal transit time
Distribution	Decreased gastric acid secretion, higher gastric pH
	Increased plasma volume
	Increased extracellular fluid
	Increased total body weight
	Decreased plasma albumin
	Respiratory alkalosis
	Increased cardiac output
Metabolism	Increased estrogens and progesterone
	Decreased CYP1A2 activity
	Increased CYP3A4 activity
	Increased CYP2D6
Elimination	Increased renal blood flow
	Increased glomerular filtration rate
	Increased creatinine clearance

Some physiologic changes are abrupt while others evolve more slowly during pregnancy. Most of the physiologic changes manifest during the first trimester and peak during the second trimester of pregnancy. Obstetric textbooks provide a more elaborate discussion of the physiology of pregnancy. Briefly, pregnancy causes changes in total body weight and body fat composition. Pregnancy may affect the bioavailability of drugs because gastric emptying is delayed [7], gastrointestinal transit time is prolonged [8], and gastric acid secretion is decreased [9]. Plasma volume expands during pregnancy with significant increases in extracellular fluid space and total body water that vary with patient weight and can affect the volume of distribution of drugs [10]. Hemodynamic changes in pregnancy include an increased cardiac output, increased stroke volume, and elevated maternal heart rate. Blood flow to the uterus, kidneys, skin, and mammary glands is increased. The percent of cardiac output attributed to hepatic blood flow is lower in pregnancy than that in the nonpregnant condition [11]. The concentration of plasma albumin decreases during pregnancy resulting in reduced protein-binding [12]. Glomerular filtration rate increases early in pregnancy and continues to rise throughout pregnancy [13]. Hepatic enzyme activity has also been reported to change during pregnancy, including CYP450, xanthine oxidase, and *N*-acetyltransferase [14, 15]. Physiologic changes are not fixed throughout pregnancy but rather reflect a continuum of change as pregnancy progresses.

The multiple physiologic changes in pregnancy provide the rationale for investigating the pharmacokinetics and pharmacodynamics during

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pregnancy. However, despite the altered physiology the assumption is often that the pharmacokinetics in pregnancy are no different from healthy volunteers and pregnant women are dosed similarly. Unfortunately there is little information available to direct appropriate prescribing for pregnant women. In the absence of information the usual adult dose is prescribed in pregnancy and may result in substantial underdosing or excessive dosages. Scientifically driven dosing recommendations derived from well-designed and well-conducted PK/PD studies are critical to the health of the pregnant woman and potentially the fetus.

Sources of Information Regarding Drug Use in Pregnancy

Before any investigator pursues studying drug kinetics in pregnancy, information regarding drug safety of that particular product will be crucial to designing a protocol and subsequently obtaining Institutional Review Boards (IRB) approval. Even though information in product labeling is usually limited, multiple other sources are available that provide comprehensive information that assess reproductive toxicities from drug exposures. For example, the on-line REPRORISK system available from Micromedex, Inc. contains electronic versions of four teratogen information databases: REPROTEXT, REPROTOX (www.reprotox.org), Shepard's Catalog [16], and TERIS [17]. These periodically updated, scientifically reviewed resources critically evaluate the literature regarding human and animal pregnancy drug exposures. Other sources of information are the more than 20 comprehensive multidisciplinary Teratogen Information Services (TIS) located in the United States and Canada, which provide patient counseling and risk assessments regarding potential teratogenic exposures (www.otispregnancy.org). Many TIS, such as MotherRisk (www.motherisk.org), employ genetic counselors, who are excellent resources for pre- and postconception counseling.

Of the thousands of pharmaceutical products available only a handful are known human teratogens [18]. Largely as a result of the thalidomideinduced birth defects, most people, both patients and clinicians, over-estimate the risk to pregnancy from drug use and perceive it to be quite large [19, 20]. The overall incidence of major malformations in the general population has been estimated at 1–5% [17]. The etiology of most congenital malformations remains uncertain; approximately 20% are caused by genetic factors and chromosomal abnormalities and 10% are caused by environmental factors such as maternal conditions (4%), infections (3%), and chemicals and drugs (approximately 1% or less) [18]. Teratogenicity is only one important aspect of drug use in pregnant women; however, the appropriate dose necessary for anticipated efficacy is critical as

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well. Sources of information on appropriate dosing in pregnancy are not available.

Methodologic Considerations

The ultimate goal of studies performed to determine the effect of pregnancy on PK/PD should be to provide useful information for appropriate dosing of drugs in pregnancy. A well-conducted study begins with a well-designed study. Studies in pregnancy may require extensive collaborative efforts that enlist the support of specialists in obstetrics, pediatrics, pharmacology, pharmacometrics, and statistics, among others.

Study Objectives

The protocol should clearly state the primary objective of the study, e.g., to determine the PK and/or PD in pregnant patients, or to determine if the PK/PD are altered in pregnant patients to such an extent that the dosage should be adjusted.

Study Participants and Control Group

The study participants optimally should be representative of the typical patient population for the drug to be studied. Consideration should be carefully given to the control group selected, and the study protocol should provide the rationale for the control group selected (Table 2).

For PK studies in pregnancy, PK parameters should optimally be compared in the pregnant and nonpregnant state with the woman serving as her own control by undergoing serial PK/PD assessments. This type of design will avoid the criticism of some PK/PD studies of pregnant women which are flawed by the comparison group selected [21, 22]. Ideally PK assessments would be done prepregnancy for baseline PK and in all three trimesters, although this is rarely practical. For chronically administered drugs an assessment of prepregnancy PK/PD could be done. When the patient becomes pregnant and if her medical condition requires that she stay on the drug of interest and the drug has a good fetal-safety profile, PK/PD assessments during pregnancy could be compared with prepregnancy. A study center that enrolls patients on chronic therapy for medical conditions prior to pregnancy would be best suited for this study design.

Many pregnant women do not seek obstetric medical care until the end of the first trimester, therefore, it may be very difficult to enroll pregnant women in the first trimester. Practical considerations limit most PK studies to the 2nd and 3rd trimesters with the baseline assessment done in the postpartum period. If the study design is such that each woman serves as her

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TABLE 2 Participant and Control Group Options and Sampling Strategies

Study	1st Trimester	2nd Trimester	3rd Trimester	Control		
				Prepregnancy	Postpartum (immediate[+])	Postpartum (remote*)
Ideal	X	X	X	X		
Optimal	X	X	X		X	X
Practical		X	X			X
Alternative						
Patient		X				
Patient			X			
Nested Pop PK[#]	X	X	X	X	X	X

[+]lmmediate assessments at 24–48 hours postpartum.
*Remote assessments at ≥2–3 months postpartum.
[#]Pop PK studies do not need to use the same patient in sequential sampling time frames.

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own control, PK/PD should be determined during the postpartum period and ideally this would include an early or remote (or both) postpartum PK/PD determination. The remote assessment should take place at least 2–3 months postpartum to allow for the physiologic changes inherent in pregnancy to return to the nonpregnant state. In addition, the women should not be lactating for the postpartum assessment to best reflect the nonpregnant state. Sometimes pharmacologic therapy needed during pregnancy will no longer be necessary in the postpartum period (e.g., hypertensive medications to control pregnancy-induced hypertension). If a drug possesses linear kinetics a single-dose postpartum PK/PD study could be extrapolated to multiple dose steady-state kinetics during pregnancy.

Consideration should be given to the inclusion and exclusion criteria and must be tailored to the study taking into account the drug and/or the disease being studied. Factors with significant potential to affect the PK/PD of a drug (e.g., the trimester of pregnancy, age, weight, diet, smoking, alcohol intake, concomitant medications, ethnicity, renal function, other medical conditions) may need to be considered as well. Uniform diagnostic criteria should be applied across pregnant patients to ensure similarity of diagnosis and also minimize drug-disease interactions that could contribute to variability. The study protocol should include the criteria for dating the pregnancy and this should be consistently applied (e.g., using last menstrual period or ultrasound for dating the pregnancy). The metabolic status should be considered for drugs that are hepatically metabolized and known to exhibit genetic polymorphisms (e.g., CYP2D6 or CYP2C19). Genotype has been shown to have an affect on pregnancy-related changes in metabolism [15].

Pharmacokinetic/pharmacodynamic studies could also be nested within a larger clinical study on safety, efficiacy, or pregnancy outcomes. For example, the PK of nifedipine was studied in a small subset of patients who were participating in a larger clinical study to assess treatment for pregnancy-induced hypertension [23].

As discussed earlier, the physiologic changes in pregnancy are dynamic and continuous throughout pregnancy and are not necessarily imminent with each trimester. In order to minimize variability for traditional PK designs, investigators should consider narrowing the time of sampling from a trimester of gestation to a "window" of gestational age. For example, the protocol could prospectively state "windows" of time for study, e.g., 20–24 weeks instead of any time in the 2nd trimester.

Sample Size

The determination of an adequate sample size depends on the objective and design of the study. Considerations for sample size should include the PK

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and/or PD variability for the drug being studied, the study design (i.e., single-dose vs. multiple-dose), and the variability of the physiologic changes inherent in pregnancy. Intraindividual and interindividual variabilities may differ in pregnancy compared with the nonpregnant state and should be considered when determining the sample size. For a population PK approach, sparse sampling with a larger number of patients may be useful as well [24].

The final number of patients enrolled may need to be in excess of the sample size calculated to take into account drop-outs or subsequent patient exclusion from the study, especially for longitudinal study designs. Some patients may be excluded from study participation in a subsequent trimester. Data for that patient will be missing for the trimester of interest; however, the patient should be continued in the study so that postpartum PK/PD assessments are done.

Sample Collection and Analysis

Consideration should be given to the type (e.g., plasma, whole blood, urine) and number of samples that are necessary to accurately estimate the relevant pharmacokinetic parameters for the parent drug and its active metabolites. Since plasma protein binding is often altered in pregnancy, total and unbound concentrations of drug and metabolites should be determined. Unbound drug concentrations are generally believed to determine the rate and extent of delivery to the sites of action. For drugs and metabolites with a relatively low extent of plasma protein binding (e.g., extent of binding less than 80%), alterations in binding due to pregnancy are most likely small in relative terms.

Data Analysis

The analysis of the study will depend on the study design characteristics. Total and unbound plasma drug/metabolite concentrations (and urinary excretion data, if collected) can be used to estimate PK parameter. The PK parameters can include the area under the plasma concentration curve (AUC), peak concentration *(C_{max})*, plasma clearance *(CL_T)* or the apparent oral clearance *(CL/F)*, apparent volume of distribution (V_z/F or V_{SS}/F), and terminal half-life *($t_{1/2}$)*. Pharmacokinetic parameters should be expressed in terms of total and unbound concentrations. For drugs and metabolites with a relatively low extent of plasma protein binding (e.g., extent of binding less than 80%), description and analysis of PK in terms of total concentrations are usually sufficient. Noncompartmental- and/or compartmental-modeling approaches to parameter estimation can be employed.

Mathematical models for the relationship between pregnancy status and relevant PK parameters can be constructed. The categorization of

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gestational age, either as a nominal (e.g., trimester) or a continuous (week of gestation) variable will direct the appropriate type of analysis. The analysis may provide an estimation of PK/PD parameters, modeling of the PK/PD relationship, and modeling of the relationship between gestational age and the PK parameters. The models selected should be adequately supported by the data and/or mechanistic arguments.

In addition, an assessment of whether dosage adjustment is warranted in pregnant patients and recommendations for dosing can be further extrapolated. Typically the dose is adjusted to produce a comparable range of unbound plasma concentrations of drug or active metabolites at baseline (prepregnancy or postpartum) compared to that during pregnancy. Simulations may identify doses and dosing intervals that achieve the goal for pregnant patients at different trimesters or gestational ages. Special statistical considerations may be necessary for longitudinal study designs given the repeated measures characteristics of the design.

Study-Design Considerations

A longitudinal study design should be considered for drugs that are administered chronically or given for several treatment cycles throughout pregnancy. In this design, pregnant women would have pharmacokinetic assessments conducted serially throughout pregnancy and each woman would then serve as her own control. The study should focus on comparing a pregnant patient at one trimester of pregnancy to the same patient at a different trimester as well as to the same patient at baseline (prepregnancy or postpartum). This type of design could potentially minimize interindividual variability throughout pregnancy.

It may be difficult to use a longitudinal study design for drugs that are given acutely (e.g., single dose or short course of therapy) in pregnancy. In such cases, a multiple-arm study design could compare different pregnant patients at different trimesters, e.g., a sample of women each in 2nd and 3rd trimesters. Each woman could again serve as her own control and have PK/PD determinations performed in the postpartum period. If it is impossible to administer drug to the same patient in the postpartum period, then an additional arm of the study using a different population of postpartum women, or female volunteers, could be used.

Ideally, the dose given for a PK/PD study in pregnancy should reflect actual clinical usage. If the drug is usually given chronically during pregnancy, multiple dosing for steady-state kinetics would be optimal. In some circumstances, the dose may need to be increased or decreased as pregnancy progresses, to achieve the appropriate therapeutic response, e.g., lowering of blood pressure, or to decrease, adverse events such as hypotensive episodes with antihypertensive therapy. In designing the study,

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investigators should consider how changes in dose over pregnancy will be handled in the analysis.

A population PK study design may also be considered. A particular advantage of the population PK approach is the assessment of multiple covariates. Techniques such as nonlinear mixed effects modeling may be used to model the relationship between covariates such as gestational age and PK parameters such as the apparent clearance of the drug *(CL/F)*. The control group selected for a population PK study design may differ from other designs and may be normal female volunteers [8].

Drug Metabolism (CYP450) Studies

Drug metabolism studies using probe substrates have been performed in pregnant women [14]. One concern about the use of probe substrates in pregnancy is the lack of direct therapeutic benefit to the pregnant woman or her fetus. For drug metabolism studies, a single dose of a probe substrate could potentially be given during pregnancy although there may be circumstances that limit dosing probe substrates in a pregnant woman. It may be reasonable to administer a probe substrate once or twice during pregnancy and once in the postpartum period for each woman in order to minimize nontherapeutic exposure to a drug. Alternatively, lower doses of probe substrates can be used in pregnancy studies.

Pharmacodynamic Assessments

Whenever appropriate, pharmacodynamic assessment should be considered when designing PK studies in pregnancy. The selection of the PD endpoints should be carefully considered and may be based on the pharmacological characteristics of the drug and metabolites (e.g., extent of protein-binding, therapeutic index, and the behavior of other drugs in the same class in pregnant patients). Similarly, biomarkers may be considered to measure PD endpoints of interest. Consideration should also be given to fetal PD assessments, e.g., fetal heart rate and rhythm response to maternal administration of an antiarrhythmic drug.

Ethical Considerations and Regulatory Framework

Ethical Considerations

Ethical considerations for studying drugs in pregnant women must be tended to in the study design and when conducting studies. Some recommend that only pregnant women who require a drug for therapeutic reasons be included in clinical studies, citing that drug studies cannot be done in normal pregnant "volunteers" [25]. Others believe that women

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should already have made the decision to use the particular drug of interest to treat a medical condition during pregnancy in order for a study to proceed. The patient should not, ordinarily, be making the decision to take the study medication in order to participate in the study. Drugs can be studied for maternal medical treatment (e.g., hypertension, seizure disorder) as well as for fetal treatment (e.g., fetal tachycardia).

Protection of Human Subjects Regulations

Studies that are supported by federal funding must comply with 45CFR46, Protection of Human Subjects [26]. Subpart A of this regulation is the basic Department of Health and Human Services Policy for Protection of Human Research Subjects, and contains basic protections for human research subjects participating in clinical research. Expedited review for studies that represent minimal risk to study subjects is possible under this regulation. Federal regulations require that IRB give special consideration to protecting the welfare of particularly vulnerable subjects, such as children, prisoners, pregnant women, mentally disabled persons, or economically or educationally disadvantaged persons. Institutional Review Board approval is necessary and ensures that risks are minimized and reasonable with benefits to subjects of study participation. Institutional Review Boards' ensure that subject selection is equitable, require informed consent for studies, review protocols to ensure safety and subject confidentiality, and ensure protection of vulnerable subjects. Many IRBs follow federal regulations on the conduct of studies in pregnant women.

Subpart B of this regulation, modified in 2001, is critical to conducting studies in pregnant women and contains additional protections for human fetuses, pregnant women, and human in vitro fertilization (Table 3).

According to Subpart B, pregnant women can give informed consent and engage in research studies if (1) studies have been conducted on animals and nonpregnant women; (2) research meets the health needs of the mother and the risk to the fetus is the minimum necessary or minimal risk; and (3) research benefits the mother, fetus, or general knowledge. In general, maternal consent is all that is necessary for the participation of pregnant

TABLE 3 Protections of Human Subjects Regulations Pertaining to Pregnant Women

Benefits of study	Consent required
General knowledge	Maternal only
Maternal health	Maternal only
Fetal health	Maternal & paternal

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women in studies. However, for studies that benefit only the fetus, both maternal and paternal consent are required for maternal participation in such studies.

Regulatory Requirements

Studies conducted under an Investigational New Drug (IND) application or with federal financial support must comply with 45CFR46 with specific attention paid to Subpart B regarding paternal consent and with 21CFR312. Studies done to support a labeling claim should comply with ICH E6, The Good Clinical Practice: Consolidated Guideline [27]. "Positive or negative experiences during pregnancy or lactation" will be one safety issue to be explicitly addressed in the Overall Safety Evaluation section of the Periodic Safety Update Report (PSUR). The International Conference on Harmonisation Guidance for Industry E2C Clinical Safety Data Management: Periodic Safety Update Reports for Marketed Drugs [28] contains more information regarding these regulatory submissions. This requirement will eventually be incorporated into the FDA Safety Reporting Regulations. Postmarketing exposure and safety data will most likely provide the appropriate background that supports the need for pharmacokinetic assessment in pregnant patients.

Incorporating PK/PD Data in Pregnancy Labeling

The current regulations regarding pregnancy labeling (21CFR 201.57 (6)(a)-(e)) promulgated in 1979 use the pregnancy categories (A, B, C, D, and X) to address teratogenic risk to the fetus from drug exposure (Table 4).

TABLE 4 U.S. Food and Drug Administration Pregnancy Labeling Categories

Pregnancy category	Category description
A	No adverse effects in humans.
B	No effect in humans with adverse effects in animals OR No effects in animals without human data.
C	Adverse effects in animals without human data OR No data available for animals or humans.
D	Adverse effects demonstrated in humans OR Adverse effects in animals with strong mechanistic expectation of effects in humans.
X	Adverse effects in humans or animals without indication for use during pregnancy.

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Prior to 1979, there was no requirement to address pregnancy in labeling. The current regulations address decision-making for the use of drugs by women who are already pregnant.

The newly proposed physician labeling rule [29] describes "pregnant women" as a special population. Unless a product has been specifically studied for an indication unique to pregnancy (e.g., treatment of preterm labor), treatment during pregnancy is not considered an "indication" for regulatory purposes. Rather, pregnant women are considered a subpopulation with altered physiology. Erroneously many health professionals and the medical literature discuss the use of drugs in pregnancy as "indicated for" or, more typically, "not indicated" for pregnancy.

Information from PK/PD studies in pregnancy should be included in product labeling. The labeling should reflect the data pertaining to the effect of pregnancy on the PK and/or PD (if known) obtained from studies conducted. Information from these studies may need to be cross-referenced to other labeling sections such as the clinical pharmacology, special populations, warnings, precautions, pregnancy, and dosage and administration sections. The FDA is working to improve the quantity and quality of data available on the use of medications during pregnancy and is in the process of revising the pregnancy labeling regulations to delete the pregnancy categories scheme and promote more useful clinical information in a narrative format [30–33].

LACTATION

Introduction

Breast milk is widely acknowledged to be the most complete form of nutrition for infants. Breastfeeding poses multiple benefits for infants including health, growth, immunity, and development. Specific infant benefits of breastfeeding include decreased episodes of diarrhea, respiratory infections, and ear infections. Breastfeeding poses multiple maternal benefits as well, including a reduction in postpartum bleeding, earlier return to prepregnancy weight, reduced risk of premenopausal breast cancer, and reduced risk of osteoporosis [34]. In order to encourage breastfeeding, the Health and Human Services "Healthy People 2010" initiative targets increasing the percentage of mothers who breastfeed to 75% in the early postpartum period, 50% at six months, and 15% at one year [35]. Professional medical organizations encourage breastfeeding as well [36, 37]. The American Academy of Pediatrics (AAP) considers breastfeeding to be

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the ideal method of feeding and nurturing infants and recommends that all women breastfeed and continue to do so until the child reaches one year of age [37].

As in pregnancy, it is highly likely that a woman will require and take medications while she is breastfeeding. Surveys in various European countries demonstrate the extent of drug use by lactating/breastfeeding women. Postpartum women who choose to breast feed take fewer medications than those who do not breastfeed [38]. Most nursing mothers (90–99%) receive a medication during the first week postpartum, 17–25% of nursing mothers take medication at four months postpartum, and 5% of nursing mothers receive long-term drug therapy [39].

When lactation studies are undertaken, the emphasis is usually on the health risk or extent of exposure in the breast-fed infant, failing to investigate maternal factors such as pharmacokinetics, dose adjustments, or other clinically relevant information that affect the efficacy or safety in breastfeeding women. Potential differences in PK might be expected in the postpartum and lactating periods due to differences in endogeonous hormones, total body weight, body fat, and muscle mass compared to nonlactating women.

Inconsistent and inadequate methodologies are often employed in lactation studies. Many studies have shortcomings such as an extremely small sample size with infrequent or single-time point sampling, thus making interpretation or comparison across studies quite difficult. The consistent application of adequate study designs should improve both the quality and quantity of data available, and assist patients and health care providers when making decisions about the use of drugs in lactating women.

The mere presence of a drug in breast milk does not necessarily indicate a health risk for the breast-fed infant. The presence or absence of the drug in milk is only the first step in determining risk. The extent of exposure to a drug in the breast-fed infant may be considerably less than anticipated by drug excretion into breast milk due to decreased bioavailability of drug in milk (e.g., tetracycline). In addition, the known or anticipated effects on the breast-fed child of drug exposure through breast milk will aid in the risk analysis. Unwarranted recommendations to stop nursing will negate the benefits of breastfeeding to both the mother and the child.

Clinical lactation studies can be designed to address different lactation issues such as PK/PD changes in lactating women, extent of drug transfer into breast milk, extent of drug transfer via breast milk to the breast-fed child, drug effect on milk (e.g., production and composition), and effects of drug exposure from breast milk on the breast-fed child. This section addresses considerations in the design of clinical lactation studies. The

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design for safety studies in the breast-fed child specifically studying the effects on the breast-fed child of drug exposure through breast milk is beyond the scope of this section.

Physiology of Lactation

Lactation is an integral part of the reproductive cycle of humans. Breast development begins in utero; however most of the morphogenesis of the breast occurs postnatally in adolescence and adulthood. Under the influence of sex steroids, especially estrogen, the mammary glandular epithelium proliferates. The breast is prepared for milk production during pregnancy through the complex endocrine changes of pregnancy, especially prolactin. Lactogenesis, the initiation of milk secretion, has been described as a three-stage process [40]. Stage I begins approximately 12 weeks before delivery and is marked by increases in lactose, proteins, including immunoglobulins, and decreases in sodium and chloride. Lactogenesis is initiated after delivery with a fall in serum progesterone, and high prolactin levels. The first milk secreted is called colostrum. This initiation of lactogenesis in Stage II does not rely on infant suckling until the third or fourth postpartum day. In Stage II, the blood flow to the breast increases. Oxygen and glucose uptake by the breast increases as does the citrate concentration. At days two and three postpartum, Stage II becomes clinically apparent with copious secretion of milk typically referred to as "the milk coming in." Major changes in milk composition continue for approximately 10 days, usually referred to as "transitional milk" and then "mature milk" is established; this final stage of lactogenesis is referred to as Stage III. The process of milk secretion requires milk synthesis and milk release.

Human milk differs from milk of other species in that the concentration of monovalent ions is lower and lactose is higher [41]. Milk contains over 200 constituents and is isosmotic with plasma. Lactose is the major carbohydrate for the milk of most species and is only found in milk. Breast milk is high in lipid most of which is long-chain fatty acids. Most proteins in milk are formed from free amino acids in the secretory cells of the breast and are specific to breast secretions [42]. Human milk contains up to 4000 white blood cells/mL and is particularly high in colostrum. Macrophages are the white blood cells found in greatest number. Mature human milk has a pH that is more acidic than plasma [43]. Human milk is not a uniform fluid but one of changing composition [44]. Milk composition differs within a given feeding with foremilk differing from hindmilk, e.g., fat content is highest in hindmilk. Colostrum differs from transitional and mature milks. Milk composition varies with maternal nutrition, the time of day, and among

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women [43]. Drugs can potentially alter the composition of breast milk including changes in protein, lactose, lipid, and electrolyte concentrations [45].

During the weaning process when milk is not removed or is less frequently removed, the increased pressure in the breast decreases blood flow and inhibits lactation. Milk protein, chloride, and sodium concentrations increase and lactose concentrations decrease during weaning. Involution of the mammary gland occurs when regular extraction of milk from the breast ceases and involves an orderly sequence of events [43]. Involution is characterized by secretory epithelial cell apoptosis, degradation of the mammary gland's basement membrane [46], and gland remodeling reverting to the prepregnant state. Involution is accompanied by a decrease in the activity for most of the enzymes involved in lipid synthesis [47]. It is not known exactly how long it takes for a lactating woman to return to her baseline status (e.g., nonpregnant, nonlactating state) after weaning is complete.

Sources of Information about Drug Transfer into Breastmilk

It is generally believed that all drugs pass into breast milk. Drugs pass into milk by simple diffusion, carrier-mediated diffusion, or active transport. Factors that influence the amount of drug that passes into breast milk include the molecular weight, protein-binding, degree of ionization, solubility, both lipid and aqueous, and the pH of plasma relative to breast milk.

There are a number of articles of drugs in breast milk including reviews and studies of a specific medication. The AAP has published consensus documents listing drugs and chemicals that are transfered into breast milk [48–50]. These publications include recommendations about drug use during breastfeeding as well. In addition, textbooks and other references are available that provide information about the use of specific drugs in breast feeding, including data of safety and drug transfer into milk [51, 52].

Many references include the milk/plasma ratio (M/P) for many drugs as an estimate of the dose of maternal drug delivered to the infant via breastmilk. The M/P ratio is the concentration of drug in the milk vs. the concentration of drug in maternal plasma (or serum). Pitfalls exist in the estimation of the M/P ratio, the most common of which is the assumption that milk and plasma drug concentrations parallel each other throughout dosing [53]. Presumed concurrence between milk and plasma drug concentrations weakens the reliability of reported data, as do M/P ratios reported from single time point determinations. PK studies in lactation must

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account for the time-dependent variation of drug concentration in milk and plasma.

Considerations for Conducting Clinical Lactation Studies

Clinical lactation studies may be undertaken to investigate PK/PD changes in lactating women. Lactation studies could also investigate the extent of drug transfer into breast milk and subsequently the extent of drug transfer into the breast-fed child. In addition, lactation studies could be designed to investigate alterations to breast milk from maternal drug exposure, such as milk volume and composition. This type of study could be done for drugs as well as larger biological molecules, especially if there is the potential to alter the composition of breast milk, e.g., vaccines and altered immunologic properties of breast milk. Finally, clinical lactation studies can be designed to investigate the effects on the breast-fed child from drug exposure via breast milk. There are many areas to consider when designing clinical lactation studies.

Methodologic Considerations

Several publications have addressed the methodologies for conducting studies on drug transfer into breast milk. A World Health Organization (WHO) Working Group published guidelines for conducting studies on the passage of drugs into breast milk [39, 54]. In addition the environmental health community has substantial experience in assessing exposures through breast milk. Some of the methodologies used in environmental health studies may be useful when designing human studies to assess exposures to Pharmaceuticals through breast milk. The WHO European Centre for Environmental and Health has been involved with monitoring environmental exposures via studies on levels of chemicals in human milk, particularly polychlorinated biphenyls (PCBs), polychlorinated dibenzo-pdioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) [55]. An expert panel discussion provided recommendations for developing a breast milk monitoring program for environmental exposures in the United States [56]. This report includes recommendations for participant selection, methods for obtaining human milk, detecting the presence of environmental chemicals in those samples, and interpreting and communicating the information found.

Study Objective

The primary objective of the study in lactating women should be clearly stated, for example, to determine if the PK and/or PD are altered in lactating

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women such that dose adjustment is necessary. Careful consideration should be given to adequate baseline determinations and comparisons to baseline. For example, for studies that are conducted to evaluate the effect on milk production (e.g., the quality or quantity of breast milk), the diurnal variation of milk production and composition should be considered in study design. Study design (e.g., participant selection, number of study subjects, sample collection) will vary according to the primary study objective.

Study Participants and Control Group

Study participants may include mother-infant pairs or lactating women alone. Optimally, the study participants would be representative of the typical patient population for the drug to be studied. Maternal factors with significant potential to affect lactation (e.g., weight, gravity, parity, stage of lactation, postpartum status, episodes and duration of previous breastfeeding) or the PK of a drug to be studied (e.g., diet, smoking, alcohol intake, concomitant medications, ethnicity, other medical conditions) should be considered. Inclusion and exclusion criteria should be carefully considered and need to be tailored to the study. Infant factors (e.g., age, term vs. preterm neonates, extent of breastfeeding, and age related changes in absorption, distribution, metabolism, and excretion) should be considered as well. Uniform diagnostic criteria should be applied to all patients to ensure similarity of diagnosis for which treatment is being given to reduce disease-specific variability in PK.

Careful consideration should be given to the control or comparison group chosen. For clinical studies, ideally the lactating woman would serve as her own control by undergoing PK/PD assessment(s) in lactation and again after weaning is complete, e.g., a longitudinal study design. The optimal control group will depend on the research question asked and the objective of the study. Potential control groups include historical controls (usually male volunteers) or female volunteers with or without the medical condition of interest. If female volunteers are used as controls, consideration should be given to matching them to study subjects (e.g., postpartum status, age). The control group should account for postpartum PK changes and identify time windows (e.g., 3–4 months postpartum) to account for variability in physiologic postpartum changes. The post weaning samples for PK/PD should be performed at similar times after weaning as well, e.g., one month after weaning in complete. The rationale for the control group selected should be provided in the study protocol.

Sample Size

Determination of an adequate sample size depends on the objective and design of the study. The number of patients enrolled in the study should be

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sufficient to detect clinically significant differences (e.g., PK differences large enough to warrant dosage adjustments). The PK variability of the drug as well as the PK/PD relationships for both therapeutic and adverse responses will affect this decision. Sample size considerations should include PK and PD variability for the drug being studied, the study design (i.e., single-dose vs. multiple-dose), and the variability in lactation physiology. Inter and intrasubject variability for mother and breast-fed child may need to be considered depending on the design and primary objective of the study. A population PK design could also be considered however practical difficulties in conducting a population PK study during lactation may limit its value. The final number of patients enrolled may need to be in excess of that originally calculated by standard sample size calculations and should take into account drop-outs and subsequent exclusion from the study.

Sample Collection and Analysis

The frequency and duration of sampling should be sufficient to accurately assess the outcome selected, e.g., estimate the relevant pharmacokinetic parameters for the parent drug and its metabolites (see Data Analysis section below). Samples should be collected in a manner to characterize the complete dosing interval. Each breast should be completely emptied at each sampling time, the volume of milk recorded, and an aliqout removed for analysis. An electric milk pump is recommended since milk composition can vary with the method used. Separate collection containers should be used for each milk collection. Pooling of different-timed milk samples is not recommended. Consideration should be given to sample handling and the protocol should include the precise details especially with milk samples (e.g., methods to minimize contamination). Total and unbound concentrations of drug and metabolites should be determined. Bioanalytical methods should determine drug and metabolite concentrations in all biological matrices studied (e.g., plasma, serum, whole blood, breast milk, urine). Milk samples should additionally be assayed for milk fat.

Data Analysis

Total and unbound plasma and milk concentration data (and urinary excretion data, if collected) can be used to estimate PK parameters of the parent drug and metabolites concentrations. Maternal PK parameter estimates can include: the area under the milk concentration curve (AUCm or AUCmilk; AUC_{0-t} or $AUC_{0-\infty}$ in single dose studies and $AUC_{0-\tau}$ at steady state), the area under the plasma concentration curve (AUCp or AUCplasma; AUC_{0-t} or $AUC_{0-\infty}$ in single dose studies and $AUC_{0-\tau}$ at steady

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state), peak concentration *(C_{max})*, time to peak plasma concentration *(t_{max})*, plasma clearance *(CL_T)* or the apparent oral clearance *(CL/F)*, apparent volume of distribution *(V_Z/F* or *V_{SS}/F)*, and terminal halflife *($t_{1/2}$)*. Pharmacokinetic parameters should be expressed in terms of total and unbound concentrations. For drugs and metabolites with a relatively low extent of plasma protein binding (e.g., extent of binding less than 80%), description and analysis of PK in terms of total concentrations are usually sufficient. As warranted by the study conducted, infant PK parameter estimates could be determined. The PK parameters of metabolites in maternal plasma, breast milk and ingested by the breast-fed infant can be estimated. If samples obtained from the breast-fed infant do not permit determination of both total and unbound (e.g., insufficient number and volume of samples), the average fraction of drug bound can be determined. Noncompartmental and/or compartmental modeling approaches to parameter estimation can be utilized.

The amount of drug or metabolite consumed by the breast-fed infant, the daily infant dosage, can be determined. The amount of drug excreted in breast milk over 24 hours was chosen arbitrarily since it represents a single day of exposure to drug via breastmilk. Any time frame could be chosen, e.g., dosing interval; however, it may be easier to interpret daily results.

The infant dosage can be calculated by summing the product of drug concentration and the volume of milk obtained at each sampling time interval:

> Daily infant dosage (mg/day)=Σ(total drug concentration in each milk collection time interval×expressed milk volume in each milk collection time interval)

Alternatively, the infant daily dose can be estimated with the following equation:

> Estimated daily infant dosage (mg/kg/day)=M/P×average maternal serum concentration×150 mL/kg/day

where M/P (milk-to-plasma ratio) is the ratio of AUC_{miik} to AUC_{plasma}, the average maternal serum concentration refers to $AUC_{0-\infty}$/dosing interval after maternal ingestion of a single dose of drug or $AUC_{0-\tau}$/dosing interval at steady state during chronic maternal dosing [39, 54]. Calculation of the M/P ratio from single paired maternal milk and plasma concentrations obtained at one sampling time is not recommended because it fails to take into account the time-dependent nature of the M/P ratio [53, 57]. The standardized milk consumption of 150 mL/kg/day, the mean milk intake of a fully breast-fed two-month-old infant, is used [39, 54, 57, 58].

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If infant dose is calculated by both the above-mentioned methods, these data should be compared and explanations sought for disparities in results.

Subsequently, the percent of the weight-adjusted maternal dose consumed in breast milk over 24 hours can be calculated:

$$\% \text{ Maternal dosage} = (\text{Infant dosage (mg/kg/day)} / \text{Maternal dosage (mg/kg/day)}) \times 100$$

Similarly, this could be calculated for a dosing interval. If the pediatric or infant dose is known (i.e., the drug is approved for pediatric use), the percent weight adjusted pediatric dose ingested can be estimated as well.

The infant serum concentration is probably the most direct measure of infant risk from a drug received from breast milk. If infant serum data are not collected, the average infant serum concentration ($C_{ss,ave}$) can be estimated by:

$$C_{ss,ave} = F \times \text{infant dosage} / CL$$

where F is the bioavailability and CL is the drug clearance in the infant, if the data are known for the pediatric population.

Study Design Considerations

When studying drugs during lactation the investigator must consider the balance and relationship between mother, breast milk, and the breast-fed child. The optimal study would evaluate all three components (e.g., mother—infant pairs); however, in some circumstances other designs can be useful (e.g., maternal milk) and may need to be performed before a mother-infant pair study is conducted. Other potential designs include only those lactating women studies which provide data on the PK of the drug in lactating women and the amount of drug transferred into breast milk. Alternatively, only women studies that provide data exclusively on milk may verify other studies (e.g., in vitro data) that predict drug transfer in human milk. In some circumstances the study of milk alone may preceed more intensive investigation utilizing mother-infant pairs.

In general mother-infant pair studies should measure the amount of drug and metabolites transferred into breast milk, characterize the PK of the drug in lactating women, and assess drug exposure in the breast-fed child via breast milk. This design would include frequent maternal blood and milk samples that are simultaneously obtained and carefully timed. This design would also include infant sampling of blood and/or urine and would encourage alternative noninvasive pediatric sampling strategies (e.g., saliva,

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tears) to reliably determine drug levels and PK parameter estimates in infants.

Clinical lactation studies could be nested within a larger clinical study on safety or efficiacy outcomes or conducted in combination with the postpartum assessment of the effects of pregnancy on PK/PD of a drug. Information obtained from single-dose studies are useful and may be more acceptable to volunteers and aid in recruitment; however, the normal therapeutic practice (e.g., dose, frequency, and route of administration) should be considered in the study design. When drugs are normally taken in repeated doses, studies performed at steady state are encouraged. For probe substrates for drug metabolism studies drugs a single dose could be given.

As with pregnancy study designs, a multiple-arm design could be used. For drugs that are given acutely (e.g., single dose or short course of therapy) it may be difficult to use a longitudinal design with the same patients throughout lactation. If there is a concern that the effects of drug use in lactation differ based upon the stage of lactation, or the postpartum status, a multiple-arm design could be considered. Each woman could serve as her own control and have PK/PD determinations performed once during lactation and after weaning is complete.

Pharmacodynamic Assessments

Whenever appropriate, pharmacodynamic assessment should be included in clinical lactation studies. The selection of the PD endpoints should be based on the pharmacological characteristics of the drug and metabolites (e.g., extent of protein binding, therapeutic index, and the behavior of other drugs in the same class in lactating patients). Similarly, biomarkers could be used to measure PD endpoints of interest. Consideration should be given to PD assessments in the breast-fed child as well, e.g., heart rate and rhythm response to maternal administration of drug.

Ethical Considerations and Regulatory Framework

Ethical Considerations

Ethical considerations for studying drugs in lactating women must be tended to in the study design and when conducting studies. Since clinical lactation studies that do not expose the breast-fed infant to drug can be done, usually the ethical hurdles are not as problematic as with pregnancy. In general, if breast-fed infants are included in clinical lactation studies, women should already have made the decision to use the particular drug of interest to treat a medical condition during breastfeeding and have made the decision to continue to breastfeed in order for a study to proceed. The

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patient should not, ordinarily, be making the decision to take the study medication in order to participate in the study.

Protection of Human Subjects Regulations

As with studies in pregnancy, lactation studies that are supported by federal funding must comply with 45CFR46, Protection of Human Subjects, and should have IRB approval. Investigators participating in studies that involve breast-fed infants should be familiar with Subpart D of this regulation regarding requirements for permission by parents or guardians (45CFR46.408) for infant participation in clinical studies.

Regulatory Requirements

A Nursing Mothers section is required in labeling (21CFR 201.57 (f) (8)); however, there are no regulations requiring that studies be performed in lactating women. The Agency has provided guidelines for the study of gender differences and states that it is medically important that a representative sample of the entire population likely to receive the drugs has been studied [59].

Labeling

As with pregnancy, the newly proposed physician labeling rule [29] describes "Lactation" as a special population; lactating women are considered a subpopulation with altered physiology. When available, information from clinical lactation studies is often included in product labeling. Information from these studies may need to be cross-referenced to other labeling sections as well. Simply indicating that "drug is present in breast milk" or reporting the M/P without the contextual setting are not very helpful for patients or prescribers. Labeling should provide clinically meaningful information to assist health care providers and their patients make decisions about drug use in lactation.

AREAS FOR FURTHER RESEARCH

Clinical pharmacology and PK studies in pregnant and lactating women can identify factors that affect drug PK, such as maternal characteristics (e.g., age, gravity/parity, race, weeks gestation), concomitant medications, or underlying medical conditions. Studies can also serve as hypothesis generating tools for further study.

In the past, stable isotopes have been used extensively for intrinsic metabolic studies; however, their use in pharmacologic studies, especially in

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pregnant or lactating women, is limited. The metabolism of glucose during pregnancy has been studied using stable isotope labeled glucose [60–63]. The idea of using an intravenous dose of a stable isotope labeled drug administered simultaneously with an unlabeled oral dose of the same drug to determine bioavailability was first introduced in 1975 [64]. No studies using stable isotopes in human pharmacologic studies have been published since 1989; however, a few investigators advocate the use of stable isotopes as a means to determine absolute and relative bioavailability in pregnant women [24, 25, 65, 66]. Studies employing stable isotopes present some potential advantages over traditional PK approaches and would decrease the number of studies necessary, decrease the biologic variation between studies (intraindividual variability), and decrease sample volume.

In addition, physiological-based PK (PBPK) modeling in animals has been utilized to predict drug transport across the placenta [67]. This type of modeling may have applicability for human pregnancy, however, animals typically used in such modeling have substantially shorter gestations compared with humans. Human pregnancy is more complicated and PBPK models designed for human pregnancy may be extremely complex. Modeling may only predict passive transport across the placenta, failing to take into account active transport processes. Physiological-based PK modeling could be further developed and validated to predict maternal PK changes resulting from pregnancy-induced physiologic changes. In vitro, animal or human placental models are useful to help predict if a drug is transferred across the placenta, as well as the extent of drug transfer, and the mechanism of transfer.

Non-clinical models (e.g., mechanistic, in vitro, animal, physicochemical-based, and PBPK) can predict the amount of drug in breast milk and may be applicable to predict infant exposures to drug in breast milk as well. The applicability and validity of nonclinical models to human lactation is still under investigation. Data obtained from clinical lactation studies can test the predictive value of the nonclinical models. The incorporation of the additional information obtained from clinical lactation studies into nonclinical models should improve the predictability of the nonclinical approaches.

New technologies for studying drug disposition may be particularly valuable in investigating gender differences in PK/PD and pharmacogentics [68]. The correlation between genetics and phenotype of drug effect in pregnancy and lactation requires further investigation and may be useful in the accurate prediction of clinical outcomes. Chronopharmacology, including chronopharmacokinetics and chronopharmacodynamics, may be important in pregnancy and lactation studies. The integration of complex information about genotype, phenotype, circadian effects, and other outcomes requires sophisticated databases, and database development may

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serve as powerful adjuncts that allow for exploration of the relationships among complex variables.

CONCLUSIONS

Many challenges are met when studying special populations such as renal or hepatically impaired patients; however, studying pregnant and lactating women presents some unique challenges. Pharmacokinetic and pharmacodynamic studies in pregnant and lactating women can assist in providing the appropriate dosage and frequency of administration in pregnancy and lactation and optimize the efficacy and safety of these products. Information drawn from scientifically conducted PK/PD studies will hopefully assist health care professionals and their patients in decision-making about the use of medications during pregnancy and lactation.

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Scientific, Mechanistic and Regulatory Issues with Pharmacokinetic Drug-Drug Interactions

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INTRODUCTION

A drug interaction implies a likely modification of the expected response to the drug in an individual, due to the exposure of the individual to one or more drugs or substances. Drug interactions which produce adverse reactions in patients are unintentional, yet drug interactions may also be intentional if they provide an improved therapeutic response or allow for a more convenient dosing regimen [1]. Drug interactions include drug-drug interactions, food-drug interactions and chemical-drug interactions, such as the interaction of a drug with alcohol or tobacco.

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In general, the frequency of possible drug interactions increases with the number of concomitantly administered drugs, multiple prescribers, poor patient compliance, patient risk factors such as predisposing illness, or advancing age. Several of these factors are interrelated. Elderly patients and patients with chronic illnesses such as hypertension or diabetes are on multiple drugs. Recent estimates show that hospital patients are concomitantly administered 7 to 12 drugs thus rendering the clinical outcome of such polypharmacy difficult to predict. Furthermore, the clinical significance and severity of a potential interaction needs to be estimated (major, intermediate, minor). For example, the interactions between ketoconazole and terfenadine, cholesterol-synthesis (CSE) inhibitors (e.g., lovastatin, simvastatin), or pimozide are being classified as major drug-drug interactions due to the foreseeable side effects and the limited therapeutic range of the drugs involved. In the case of terfenadine or pimozide administered together with imidazol or triazol antimycotics, a prolongation of the QT-interval, ventricular tachycardia (Torsades de pointes) with loss of consciousness, and perisystole have been reported [2]. A combination of ketoconazole or itraconazole with CSE-inhibitors may result in severe myalgia and myopathia and may ultimately lead to rhabdomyolysis, a loss of skeletal muscle mass. On the other hand, the combination of ketoconazole with Cyclosporin A and certain benzodiazepines (e.g., midazolam, triazolam) has been categorized into the intermediate severity class. In the case of Cyclosporin A therapeutic drug monitoring and monitoring of kidney function has been recommended, whereas with oxidatively biotransformed benzodiazepines, a reduction of their dose needs to be considered or alternatively, a benzodiazepine which is not eliminated by oxidative biotransformation is recommended. The decrease of the bioavailability of ketoconazole by concomitant administration of H2-antihistamines has been termed a minor interaction [2]. This interaction is due to the dependence of dissolution of ketoconazole upon gastric pH and an increase in gastric pH will ultimately lead to a reduction of the dissolution rate of ketoconazole. This interaction can be avoided, if the H2-antihistamines are dosed two hours before or six hours following the dosing of ketoconazole.

This chapter provides an overview of the different mechanisms by which pharmacokinetic drug-drug interactions occur and an overview of the regulatory considerations with regard to the study of drug-drug interactions from the U.S. Food and Drug Administration, the European and the Canadian health authorities' perspectives. Finally, the role of the population screen in the study of possible drug interactions in phase III clinical trials will be briefly outlined.

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DRUG-DRUG INTERACTION MECHANISMS

Pharmacokinetic drug-drug interactions are commonly classified according to whether they occur during the absorption, the distribution, the metabolism, or the elimination phase (ADME). An alternative—mechanistic—classification scheme groups drug-drug interactions into:

i. drug-drug interactions based on the reaction with one or more macromolecules
ii. physicochemical interactions and interactions based on changes in local pH and, connected therewith, changes in the ionization state of molecules
iii. based on pharmacodynamic mechanisms.

Drug-Drug Interactions with Involvement of Macromolecules

Drug-drug interactions with the involvement of macromolecules are based on either the blockade of binding sites of one drug by a competing drug, or generally, the change in binding behavior of a drug to a macromolecule in the presence of an interacting molecule, or a change in the amount of macromolecules present (e.g., an increase of drug metabolizing enzymes in the presence of enzyme-inducing drugs).

Macromolecules that are important contributors of a drug-drug interaction can be drug-metabolizing enzymes, which catalyze phase I or phase II metabolic reactions, resulting in the formation and elimination of pharmacologically active and/or inactive metabolites. Furthermore, a drug-drug interaction can take place as a result of an interaction of drugs with one or more, transporter proteins, which may be critical for the passage of drugs across biological membranes. This process is sometimes also being referred to as phase III of drug metabolism. In this particular case, the excretion of a polar—membrane impermeable—metabolite from the intracellular compartment in which it has been formed, is enhanced by binding to and subsequent transport by a membrane-bound transporter macromolecule. Finally, plasma proteins are to be mentioned, which may be viewed as a high-capacity reservoir of drugs in plasma. The significance of drugs contained within the reservoir is that they are in that state neither pharmacologically active, nor do they undergo significant clearance processes.

Biotransformation-based Pharmacokinetic Interactions

A number of prominent drug products have been withdrawn in recent years because of severe drug-drug interactions and despite preclinical safety

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assessment. Mibefradil, a novel calcium antagonist, for example, was approved in Switzerland in 1996 and was also launched in the U.S. in 1997 as well as in several other European countries. Shortly following its launch as an antihypertensive and antianginal agent, reports about serious pharmacokinetic and pharmacodynamic interactions with other drugs frequently administered to patients with cardiovascular diseases were noted. These interacting drugs are to a great extent metabolized by Cytochrome P_{450} (CYP_{450})-dependent microsomal enzymes, including widely prescribed drugs like quinidine, digoxin, cyclosporin A, terfenadine, and metoprolol. In addition, reports on severe rhabdomyolysis in patients on mibefradil who were simultaneously receiving lovastatin or simvastatin were issued. Mibefradil was reported to mainly inhibit CYP2D6 and 3A4 isoenzymes. In 1998 the drug was withdrawn from the market due to the information gathered about the severity of drug-drug interactions in patients receiving mibefradil and other medications [3]. Another example of clinically important interactions between CYP3A4 inhibitors and drugs largely eliminated by oxidative biotransformation is between ketoconazole, itraconazole, clarithromycin, erythromycin, nefazodone, and ritonavir as inhibitors, when these are coadministered with terfenadine, astemizole, cisapride, or pimozide.

In that case, Torsades de pointes, a life-threatening ventricular arrhythmia associated with QT prolongation has been shown to occur as a consequence of decreased clearance of the arrhythmia-causing parent compound or metabolite [4]. Finally, a drug-drug interaction between sorivudine, an antiviral drug, and 5-fluorouracil, an anticancer drug, caused one of the most serious cases of toxicity ever seen in Japan. The interaction is based on the irreversible inhibition (mechanism-based inhibition) of dihydropyrimidine dehydrogenase, a rate limiting enzyme in the metabolism of 5-fluorouracil by a metabolite of sorivudine, which is formed by gut flora [5]. On the basis of these case reports on drug-drug interactions due to decreased metabolic clearance of the active compound and the clinical experience, several recommendations have been made for the regulatory assessment of new active substances with respect to drug-drug interactions. These include the requirement for a detailed understanding about the mechanism of biotransformation of the parent compound and its metabolites primarily by in vitro studies with human liver enzymes in which the potential for metabolic interactions with other drugs is outlined. This first screen then may serve as a start for identification of drugs that are commonly used in the target population and that may represent a particular risk by pharmacoepidemiological studies. Here, particular attention is to be put on drugs with "a high first-pass metabolism" and "a narrow therapeutic index." These may then be studied in interaction studies in the patient population or in healthy volunteers before their introduction into clinical

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practice. Particular attention needs to be put on the interpretation with respect to the severity of a drug-drug interaction. Here, not only the mean of the interaction effect, but also the observed and the theoretically conceivable extreme effects in individual subjects need to be addressed. In particular, the mibefradil case has shown that for drugs that are expected to be co-administered in the target population and that may represent a particular risk, a labelling in the product information indicating the possibility of an interaction should not be acceptable as a substitute for performing the appropriate interaction studies before introduction of the new drug into clinical practice.

Biotransformation-based drug-drug interactions may occur presystemically, i.e., at the level of the intestine and in the liver (gastrointestinal and hepatic first-pass effect) and thus may affect the bioavailability and the clearance of a drug. The intrinsic organ clearance is defined as:

$$Cl_{\text{int}} = \sum_{i=1}^{N} \frac{V_{\max,i}}{K_{m,i}}$$

where $V_{\max,i}$ and $K_{m,i}$ are the maximum reaction velocity and substrateenzyme affinity constant for the ith enzyme. Drug-drug interactions may affect intrinsic clearance. In the case of competitive enzyme inhibition, K_m is increased, whereas for noncompetitive inhibition, a decrease in $V_{\max}$ is noted. Enzyme induction, on the other hand, results in an increase of $V_{\max}$. In particular, for low hepatic extraction drugs (E<0.2), clearance is primarily dependent upon intrinsic clearance (enzyme activity) and not liver blood flow. Consequently for these drugs, small changes in intrinsic clearance, e.g., due to enzyme induction or inhibition, may result in severe changes of drug clearance. On the other hand, high hepatic-extraction drugs (E>0.6) have an intrinsic hepatic clearance which exceeds the hepatic blood flow. Clearance of these drugs is therefore primarily dependent on liver blood flow and not on intrinsic hepatic clearance. High ratios of the area under the curves in the presence and absence of an inhibitor are to be expected when the value of $(1+I/K_i)$ is large, i.e., at high concentrations of a high affinity inhibitor, and/or when the fraction of the dose eliminated by a pathway which can be inhibited by the metabolic inhibitor is large. A particular issue is the relevance of I and K_i values for the likelihood of an in vivo drug-drug interaction. In the case of reversible inhibition, a drug-drug interaction (potential for in vivo inhibition) is considered "highly likely," if K_i<1 μM and I/K_i>1 [6]. When K_i is between 1 and 50 μM and I/K_i equals 0.1–1, an in vivo interaction is deemed possible, and when K_i>50 μM and I/K_i<0.1 the potential of an in vivo

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interaction is rather remote. Consequently, if the I/K_i value is larger than 0.3–1, it has been suggested to consider designing the appropriate in vivo drug interaction studies [7]. The principle has been depicted again schematically in Fig. 1. It needs to be pointed out though, that the zone of medium risk is a gray zone and the definition of universal cut-off values is not uniquely agreed upon by several researchers. Nevertheless, high I/K_i values for a particular metabolic pathway suggest that the possibility of occurrence of a drug-drug interaction in vivo because it is likely that the inhibitor also inhibits other metabolic pathways which have not been identified yet. For mechanismbased inhibition, K_i values<20 μM for the inhibitor have "likely" potential for in vivo inhibition, whereas K_i values in the range of 20 to 100 μM and >100 μM have "possible" and "remote" potential for causing an in vivo interaction, respectively. The principle has successfully been applied e.g., for the prediction of the absence of an interaction between warfarin and tenoxicam, both of which are eliminated by CYP2C9 [8]. Similarly, an in vivo interaction has been predicted between warfarin and lornoxicam [8], tolbutamide and sulfaphenazole, and triazolam and ketoconazole [7]. For the CYP2D6-mediated dehydration of sparteine and the interaction with the CYP2D6 inhibitor quinidine, the interaction between the CYP1A2 inhibitor ciprofloxacin and the CYP1A2 substrate caffeine, and the CYP3A4 substrate cyclosporin and the CYP3A4 inhibitor erythromycin as well as for the interaction between the CYP3A4 substrate terfenadine and the CYP3A4

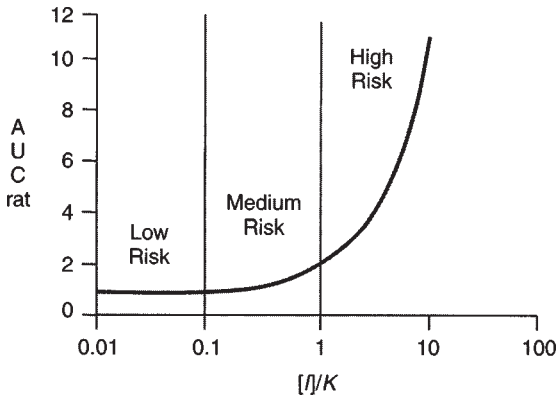

FIGURE 1 Impact of *[I]/Ki* on the ratio of the AUC of substrate ([S]<K_m)in the presence and absence of a competitive inhibitor. The equation governing the relationship is: AUC*i*/AUC=1+*[I]/Ki,* where AUC*i* and AUC are the areas under the substrate concentration-time curve in the presence and absence of the inhibitor, respectively. The figure was redrawn according to Tucker et al. [14].

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inhibitor ketoconazole, the magnitude of the interactions was underpredicted by factors of approximately 2, 1.5, 1.3, and 7, respectively [7]. The reasons for this underprediction may include estimation errors for K_i, the possibility that other elimination pathways may also be reduced by the inhibitor and the possible accumulation of the inhibitor in the liver. The latter leads to an underprediction of the inhibitor concentration at the site of metabolism, which may be the case when carrier-mediated transport processes promote the uptake of the inhibitor into hepatocytes, e.g., in the case of ciprofloxacin.

How to predict inhibitory effects of co-administered drugs on hepatic metabolism of other drugs?

The procedure for predicting the metabolic inhibition by one drug that is expected to be administered together with the study drug involves several steps. First, the metabolic pathway of the drug under consideration and possibly the P450 isozyme(s) most relevant for its degradation should be identified. This can be done either from metabolic pharmacokinetic drug interaction databases [9] or it can be determined experimentally e.g., by human P450 expression systems or by inhibition studies with human liver microsomes using P450 antibodies or inhibitors specific for each isozyme. A list of P450 isozymes and their inhibitors is given in Table 1. Secondly, pharmacokinetic data for the co-administered drug that possibly inhibits the isozyme responsible for the metabolism of the study drug are assembled and the maximum concentration of the co-administered inhibitor is estimated. Thirdly, the K_i of the inhibitor for the metabolism of the study drug is determined using e.g., human liver microsomes or human P450 expression systems and the I/K_i ratio is calculated. For more detailed information on in vitro metabolic methodology, see Chapter 5.

In addition to the selection of a particular in vitro model, particular probe substrates and inhibitors have to be chosen for the drug-drug interaction study. Table 1 is a compilation of suitable compounds for each of the human CYPs. These compounds currently present the most useful tools to provide in vitro enzyme-kinetic parameters with respect to the various CYP isoforms [10]. For a variety of reasons, e.g., not approved as a drug product and/or toxicity in humans, several of the compounds listed in Table 1 are not suitable for in vivo drug-drug interaction studies in humans. Therefore, Table 2 contains a list of probe substrates and inhibitors of CYP isoenzymes which may be used for in vivo studies in humans. The conduct of in vivo studies is most relevant to confirm positive outcomes of drug-drug interactions from in vitro findings and cases are known, in which compounds prove to be potent inhibitors of CYP isoenzymes in vitro in liver microsomes, yet have no inhibitory effect on the AUC of various probe substrates in vivo. This may, for example, be explained by the fact that microsomes are poor

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TABLE 1 In vitro Probe Substrates and Inhibitors for CYPs

CYP	Substrates	Inhibitors
1A2	Ethoxyresorufin, Phenacetin, Caffeine, Theophylline, Acetanilide, Methoxyresorufin	Furafylline, α-Naphthoflavone[1]
2A6	Coumarin	Coumarin
2B6	S-Mephenytoin, Bupropion	Sertraline[2]
2C8	Paclitaxel	Troglitazone[3]
2C9	S-Warfarin, Diclofenac, (Tolbutamide)	Sulphaphenazole
2C19	S-Mephenytoin, Omeprazole	Ticlopidine,[2] Nootkatone[2]
2D6	Bufuralol, Dextromethorphan, Metoprolol, Debrisoquine, Codeine	Quinidine
2E1	Chlorzoxazone, 4-Notrophenol, lauric acid	4-Methyl pyrazole
3A4	Midazolam, Testosterone, Nifedipine, Felodipine, Cyclosporine, Terfenadine, Erythromycin, Simvastatin, Tacrolimus	Ketoconazole, Troleandomycin, Tacrolimus, Cyclosporin

[1]Can also activate and inhibit CYP3A4.
[2]Also inhibits CYP2D6.
[3]Also inhibits CYP2C9.

TABLE 2 In vivo Probe Substrates and Inhibitors for CYPs

CYP	Substrates	Inhibitors
1A2	Caffeine, Theophylline	Enoxacin, Ofloxacin
2B6	Bupropion	Sertraline[2]
2C8	Paclitaxel[1]	Troglitazone[3]
2C9	Tolbutamide, Flurbiprofen, Diclofenac, Phenytoin	Sulphaphenazole, Fluconazole[4]
2C19	Mephenytoin, Omeprazole	Ticlopidine
2D6	Debrisoquine, Dextromethorphan, Metoprolol, Desipramine, Codeine	Quinidine
2E1	Chlorzoxazone	Disulfiram
3A4	Midazolam, Erythromycin, Simvastatin, Atorvastatin	Grapefruit juice, Ketoconazole,[5] Itraconazole

[1]Cannot be administered to healthy volunteers.
[2]Also inhibits CYP2D6 at high doses exceeding 150 mg/day.
[3]Also inhibits CYP2C9.
[4]Also moderately inhibits CYP3A4.
[5]Also an inhibitor of 2C19.

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performers with respect to phase II metabolic reactions and the scavenging of potentially inhibitory phase I metabolites is not an issue in whole functional hepatocytes. An alternative to the use of very specific enzyme inhibitors in clinical studies is the application of inhibitors with broad inhibition specificity. Examples include Cimetidine (3A4, 2D6, 1A2, 2C9) and Ritonavir (3A4, 2D6, 2C9, 2C19). Furthermore, genetic polymorphisms need to be taken into account. The polymorphic variability of drug metabolism was empirically recognized before the P450 system was well understood. Slow and rapid acetylators of isoniazid were recognized in the 1950s. Glucose-6-phosphate dehydrogenase deficiency leading to hemolytic anemia was appreciated as a genetically based variation in drug metabolism. In the 1970s, Ziegler and Biggs [15] noted that African-American patients had significantly higher nortriptyline levels than did other patients, and these investigators assumed there were genetic differences [11]. The differences in nortriptyline metabolism are now believed to result from genetic polymorphisms related to 2D6, 2C9, and/or 2C19. CYP P450 polymorphisms known today are tabulated in Table 3. Due to very active research in this field, in particular in the area of genotyping or phenotyping of individuals with respect to P450 enzymes, it is expected that this list will continue to grow. Taking the information on the different metabolism capacities of individuals it may thus be possible to predict that only those individuals in whom a major metabolic pathway is inhibited may show profound drug-drug interactions. On the other hand, the same drug combination may be estimated as having no interactions, when administered to a subject who is genetically deficient with respect to the isoenzyme responsible for drug clearance.

In addition to enzyme inhibition, induction processes by some xenobiotics, both drugs and environmental substances such as cigarette smoke, may increase the synthesis of P450 proteins. This induction process may lead to decrease in circulating plasma levels of the parent drug administered and increase in the concentrations of metabolites produced and is one of the major underlying mechanisms for time-dependent pharmacokinetics. For example, co-administration of the potent inducers rifampin or nevirapine [12, 13], and methadone has led to opiate withdrawal symptoms. Cytochromes P450 3A4, 1A2, 2C9, 2C19, and 2E1 may all be induced. Important inducers are e.g., carbamazepine, oxcarbazepine, phenytoin, phenobarbital, rifampin, rifabutin, nevirapine, troglitazone, dexamethasone, prednisone, St. John's wort, and primidone (3A4), tobacco smoke, brussel sprouts, broccoli, cabbage and other cruciferous vegetables, charbroiled foods, e.g., burned meats (1A2), rifampin, phenytoin, secobarbital (2C9), rifampin (2C19), alcohol, and isoniazid [14–17].

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TABLE 3 CYP P450 Polymorphisms [Cozza, Armstrong, 2001]

CYP	Polymorphisms	Type(s)	Phenotype substrate probe	Examples of drugs that warrant caution for PMs
2D6	Yes	PM, EM, UEM	Debrisoquin, dextromethorphan, sparteine	Antiarrhythmics, codeine, tramadol
3A4	Possible	Unknown	Cyclosporine, erythromycin, lidocain, midazolam	Unknown
1A2	Probable	Unknown	Caffeine, theophylline	Unknown
2C9	Yes	PM, EM	Naproxen, phenytoin, tolbutamide	S-Warfarin
2C19	Yes	PM, EM	Proguanil	Cyclophosphamide, ifosfamide
2E1	Yes	Unclear	Chlorzoxazone	Anesthetic agents

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Numerous examples of documented and clinically relevant drug-drug interactions exist with respect to enzyme induction. For example, induction of 3A4 by oxcarbazepine can induce the metabolism of oral contraceptives rendering them less effective [18]. Plasma concentrations of mirtazapine, a nonadrenergic and specific serotonergic antidepressant which is mainly metabolized by CYP 2D6 and CYP 3A4 are decreased by 60% following enzyme induction by carbamazepine [19]. Rifampicin and rifapentine induction can decrease plasma concentrations of protease inhibitors or non-nucleoside reverse transcriptase inhibitors which may lead to viral resistance (decreased sensitivity to the protease inhibitor or NNRTIs). St. John's wort was recently found to decrease mean trough plasma concentrations of indinavir by 81% (20), cyclosporine A by 43% (21), digoxin AUC by 25%, and trough concentrations by 33% (22). Interestingly, the effect of St. John's wort was only seen following chronic dosing of the hypericum extract and not after single dose, indicating that the mechanism of action is by induction of protein expression and not by direct competition with the concomitantly administered drug.

Transporter-based Pharmacokinetic Interactions

In addition to clearance via phase I or phase II biotransformation processes, elimination of parent xenobiotics and/or their phase I and phase II metabolites from the systemic circulation may also be driven by carriermediated transport. Transporter-related elimination of polar phase II metabolites has at times been referred to as phase III of drug elimination [23], although the same terminology has been applied to the metabolism of phase II metabolites as well, i.e., deconjugation reactions leading to the reformation of parent compound. Carrier-mediated transport is particularly important for molecules that would otherwise not be able to permeate across biological membranes, in particular due to limitations in their size, charge, or polarity. The liver, the kidneys, and the intestine are housing the majority of drug transporters responsible for carrier-mediated drug elimination. In addition to elimination processes, membrane transporters are having important functions in the organ distribution and absorption of several drugs. This is schematically depicted in Fig. 2. According to the human genome project, the estimated number of human protein-encoding transcripts approximates 30,000 with 26,588 genes showing strong corroborating evidence [24]. Out of these, 533 are transporters for inorganic and organic matter. Most transporters are organized in one out of two superfamilies. These are the "solute carrier superfamily", SLC and the "ATP binding cassette," ABC superfamily. Currently, 212 genes are family members of the SLC super family including isoforms, member-like, and antisense sequences as given by the

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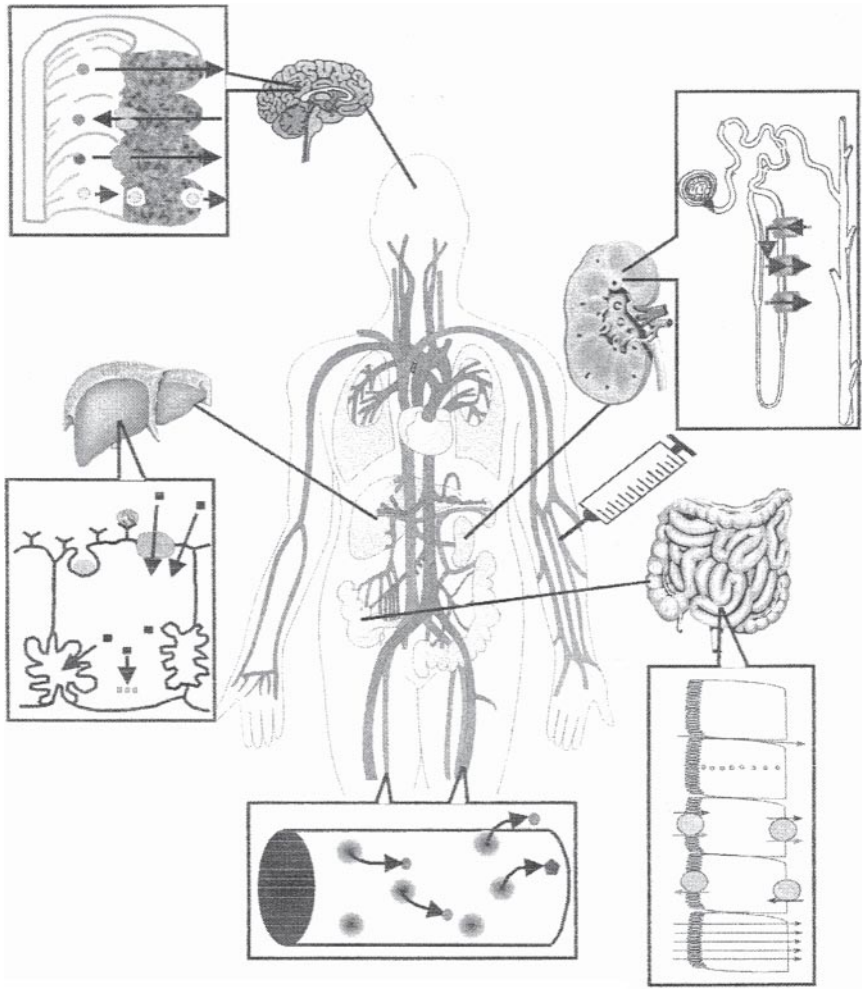

FIGURE 2 Schematic tissue distribution and function of some membrane transporters with respect to disposition and absorption of drugs.

family resources page [25]. A gene is being defined as a DNA segment that contributes to phenotype/function. In the absence of demonstrated function a gene is characterized by sequence, transcription, or homology [26]. Table 4 lists the families of known members of the SLC superfamily together with a selection of their proposed ligands. Interestingly, only three out of a total of 32 families are intracellular transporters, the vast

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majority is localized at the plasma membrane of cells. Out of these, only a subset of transporter families is believed to be of relevance with respect to the transport of drug molecules. These are pointed out in Table 4 and specific examples of drugs/xenobiotics are given. With respect to the substrate recognition patterns, the terminology on transporters is sometimes confusing. For example, members of the solute carrier family 22 include organic cation transporters (OCT1, OCT2, OCT3, OCTN1, OCTN2) but also anion transporters (OAT1, OAT2, and OAT3). Thus, members of this family are able to recognize both positively and negatively charged drug molecules. The second superfamily (ABC family) includes 48 transporter genes. The subfamilies, their names, the number of transporters in each subfamily, and a selection of proposed ligands are given in Table 5. The term "ABC" (ATP binding cassette) stems from the fact, that all members of the ABC superfamily are primarily active transporters, i.e., the energy for the directed transport of the substrate is supplied by ATP hydrolysis. "Active transport" is generally characterized by the requirement for energy, substrate specificity, preferential transport direction, saturability, and competitive inhibition by cotransported molecules. The term "facilitated diffusion" on the other hand describes a process in which the carrier-mediated transport step is not directly coupled to an energy providing source (most of the SLC transporters). The driving force is rather provided by an electrochemical gradient across the membrane (secondary active transport), which is generated, e.g., by the unequal distribution of positively or negatively charged ions (Na^+, H^+, HCO_3^-, Cl^-) across the membrane.

Carrier-mediated absorption, distribution and elimination processes have in fact been known for a long time. Some examples of substrates for carrier-mediated absorption are D-glucose, L-dopa, iron (Fe^{2+}), ascorbic acid, small peptides, penicillins, cephalosporins, angiotensin-converting enzyme inhibitors, and gabapentin, as has been recognized for some time. With respect to carrier-mediated distribution, selected uptake into and exclusion from the blood-brain barrier has been described for e.g., L-dopa, D-glucose, L-phenylalanine, asimadoline, cyclosporin A, digoxin, colchicine, vinblastine, and amitriptyline. Furthermore, placental drug passage has been described to be modified by membrane carrier proteins that are expressed in the maternal-facing brush-border membrane and the fetalfacing basal membrane of the syncytiotrophoblast, the polarized epithelium, and the functional unit of the placenta. Examples include digoxin [27], valproic acid [28], monoamines (serotonin, dopamine, amphetamine, imipramine), clonidine, cimetidine, and amiloride as well as cephaloridine [29]. In addition, various transporters for monocarboxylates (MCT1, 3, 5, 5, and 7) and dicarboxylate have been found. A summary of characterized transporters expressed in placenta is given by Ganapathy [30].

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TABLE 4 Family Members of the SLC Superfamily of Solute Transporters

Family	Members	Names	Drug/xenobiotic ligands
SLC 1	7	Glutamate transporter; Aspartate/glutamate transporter	
SLC 2	16	Facilitated glucose transporters; facilitated glucose/fructose transporters	
SLC 3	2	Cystine, dibasic and neutral amino acid transporters	L-dopa
SLC 4	13	Anion exchangers, Na^+ bicarbonate cotransporters	
SLC 5	7	Na^+/glucose cotransporters, inositol transporter, sodium iodide transporter, sodium-dependent vitamin transporters, choline transporter	
SLC 6	16	Neurotransmitter transporters including GABA, noradrenalin, dopamine, serotonin, glycine, taurine, L-proline, creatine, betain	
SLC 7	11	Cationic amino acid transporters, y+ system	
SLC 8	3	Na^+/calcium exchangers	
SLC 9	10	Na^+/hydrogen exchanger	
SLC 10	2	Na^+/bile acid cotransporters	
SLC 11	3	Proton-coupled divalent metal ion transporters	
SLC 12	8	Na^+/potassium/chloride transporters	
SLC 13	4	Na^+/sulfate and Na^+/dicarboxylate transporters	
SLC 14	2	Urea transporters	
SLC 15	2	Oligopeptide transporters, H^+/peptide transporters	β-lactam antibiotics, ACE inhibitors
SLC 16	10	Monocarboxylic acid transporters	Benzoate, lactic acid, short chain fatty acids
SLC 17	7	Na^+ phosphate transport/cotransport	PAH, Faropenem, penicillins
SLC 18	3	Vesicular monoamine, acetylcholine transporters	

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SLC 19	3	Folate transporter, thiamine transporter	Methotrexate
SLC 20	2	Phosphate transporter	
SLC 21	14	Organic anion transporter, prostaglandin transporter	Fexofenadine, β-lactam antibiotics, pravastatin, BSP, prostaglandins
SLC 1	7	Glutamate transporter; Aspartate/glutamate transporter	
SLC 2	16	Facilitated glucose transporters; facilitated glucose/fructose transporters	
SLC 3	2	Cystine, dibasic and neutral amino acid transporters	L-dopa
SLC 4	13	Anion exchangers, Na^+ bicarbonate cotransporters	
SLC 5	7	Na^+/glucose cotransporters, inositol transporter, sodium iodide transporter, sodium-dependent vitamin transporters, choline transporter	
SLC 6	16	Neurotransmitter transporters including GABA, noradrenalin, dopamine, serotonin, glycine, taurine, L-proline, creatine, betain	
SLC 7	11	Cationic amino acid transporters, y+ system	
SLC 8	3	Na^+/calcium exchangers	
SLC 9	10	Na^+/hydrogen exchanger	
SLC 10	2	Na^+/bile acid cotransporters	
SLC 11	3	Proton-coupled divalent metal ion transporters	
SLC 12	8	Na^+/potassium/chloride transporters	
SLC 13	4	Na^+/sulfate and Na^+/dicarboxylate transporters	
SLC 14	2	Urea transporters	
SLC 15	2	Oligopeptide transporters, H^+/peptide transporters	β-lactam antibiotics, ACE inhibitors
SLC 16	10	Monocarboxylic acid transporters	Benzoate, lactic acid, short chain fatty acids
SLC 17	7	Na^+ phosphate transport/cotransport	PAH, Faropenem, penicillins
SLC 18	3	Vesicular monoamine, acetylcholine transporters	
SLC 19	3	Folate transporter, thiamine transporter	Methotrexate

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TABLE 4 Continued

Family	Members	Names	Drug/xenobiotic ligands
SLC 20	2	Phosphate transporter	
SLC 21	14	Organic anion transporter, prostaglandin transporter	Fexofenadine, β-lactam antibiotics, pravastatin, BSP, prostaglandins
SLC 22	13	Organic cation transporters	TEA, dopamine, PAH, NSAIDs, penicillins, cimetidine, quinidine, verapamil
SLC 23	2	Nucleobase transporters	
SLC 24	4	Na^+/potassium/calcium exchanger	
SLC 25	19	Mitochondrial transporters	
SLC 26	10	Sulfate transporters	
SLC 27	6	Fatty acid transporters	Long chain fatty acids
SLC 28	3	Na^+-coupled nucleoside transporters	Nucleosides and analogues
SLC 29	2	Nucleoside transporters	Nucleosides and analogues
SLC 30	4	Zinc transporters	
SLC 31	3	Copper transporters	
SLC 32	1	GABA vesicular transporter	

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TABLE 5 Family Members of the Human ABC Superfamily of Solute Transporters

Subfamily	Members	Names	Drug/xenobiotic ligands
ABCA	12	ABC1	Estramustin
ABCB	11	MDR	Steroids, hydrophobic (basic) compounds, peptides, phosphatidylcholine, iron, bile salts
ABCC	12	MRP	Anionic conjugates with glutathione, with sulfate and with glucoronosyl or GSSG and X + GSH, e.g., glucuronosyl bilirubin, bile salts, nucleoside-based antivirals (e.g., azidothymidine), organic anions, nucleotide analoga (e.g., 6-mercaptopurine, thioguanine), cyclid nucleotides (3′,5′-cyclic GMP, inhibitable by sildenafil).
ABCD	4	ALD	Long chain fatty acids
ABCE	1	OABP	
ABCF	3	GCN20	
ABCG	5	White	Chemotherapeutic drugs, plant sterols

Carrier-mediated excretion in the liver and/or kidney and/or gastrointestinal tract has been described for *p*-amino-hippuric acid, penicillins, cephalosporins, digoxin, doxorubicin, fluvastatin, lovastatin, vincristine, quaternary ammonium compounds, ciprofloxacin, and indocyanine green. Relatively new is the knowledge of carrier-mediated anti-absorptive transporters in the intestine. It has been difficult for some time to differentiate between intestinal exsorption and metabolism, since both may reduce the amount entering the portal blood in a dose-dependent manner leading to low bioavailability at the low dose level and higher bioavailability at higher dose levels. Differentiation in in vivo studies—on the clinical level—appears to be possible only by selective inhibition using process-specific inhibitors [30, 31].

Intestinal exsorptive transporters became evident as soon as highly specific, potent, and low-dosed drugs were developed. Limited peroral bioavailability or lack of bioavailability of various newly developed compounds were indicative of bioavailability-limiting processes. Likewise, carrier-mediated transport across the membranes of the blood-brain barrier, the kidney, and the liver were recognized for some time to contribute to the distribution and clearance of a drug from the systemic circulation.

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Carrier-mediated absorptive or exsorptive processes are saturable and inhibitable. In Fig. 3, the relationship between the administered dose and the bioavailability or fraction dose absorbed for saturable exsorptive and absorptive processes is demonstrated schematically. Each transporter may be characterized with respect to K_m and V_{max}, i.e., regarding the substrate concentration at which half-maximal transport velocity and maximum transport rate are observed. The overall contribution of the respective transport processes in the absorption of the drug determines the relevance of the saturable mechanism. High passive permeabilities significantly reduce the relevance of carrier-mediated inside- or outside-directed transport processes, although the affinities of the substrate to the respective transporters may be high. Similarly to drug metabolism, in principle, two different types of drug-drug interaction mechanisms are feasible with respect to compounds, which are substrates for transporters:

a. Inhibition (reduction) or enhancement of drug transport through competitive/noncompetitive inhibition of binding, or transport or increase of transport through interaction with the transporter, and

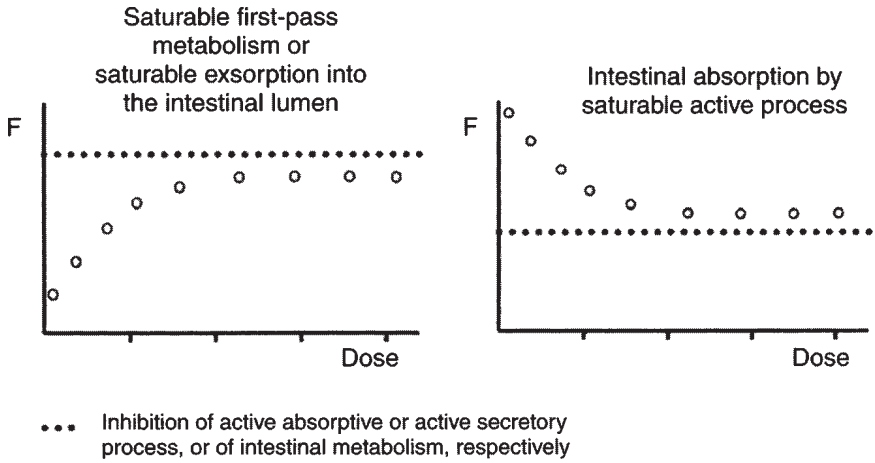
Saturable first-pass metabolism or saturable exsorption into the intestinal lumen
F
Dose
Intestinal absorption by saturable active process
F
Dose
Inhibition of active absorptive or active secretory process, or of intestinal metabolism, respectively

FIGURE 3 Potential for drug-drug and drug-food interactions at the presystemic level: First-pass metabolic or secretory processes on the one hand, or absorption via an active process on the other hand may cause nonlinearities of the bioavailability vs. dose relationship. The relative contribution of the saturable process is reduced upon increasing dose. Inhibition of the respective metabolic or transport process leads to a partial or complete disappearance of nonlinearity.

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b. Alteration of transporter expression, i.e., changes of the number of protein molecules available for the transport of drugs (induction or reduced expression).

Most relevant in this respect appear to be transport inhibition and induction of transporter expression. Transport inhibition in the intestine, for example, leads to a decrease of bioavailability, when an inside-directed transport process is reduced or inhibited. Figure 4 illustrates the changes in transepithelial drug flux based on concomitant absorption via a carriermediated process, passive diffusion, and exsorption (secretion), when competitive inhibition occurs affecting active, yet not passive transport processes. Inhibition of secretory transporters leads to enhanced drug flux across the membrane in the absorptive direction (Fig. 5). On the other hand, in the case of induction of secretory transporters (Fig. 6), transepithelial apical to basolateral fluxes decrease as soon as the expression of the

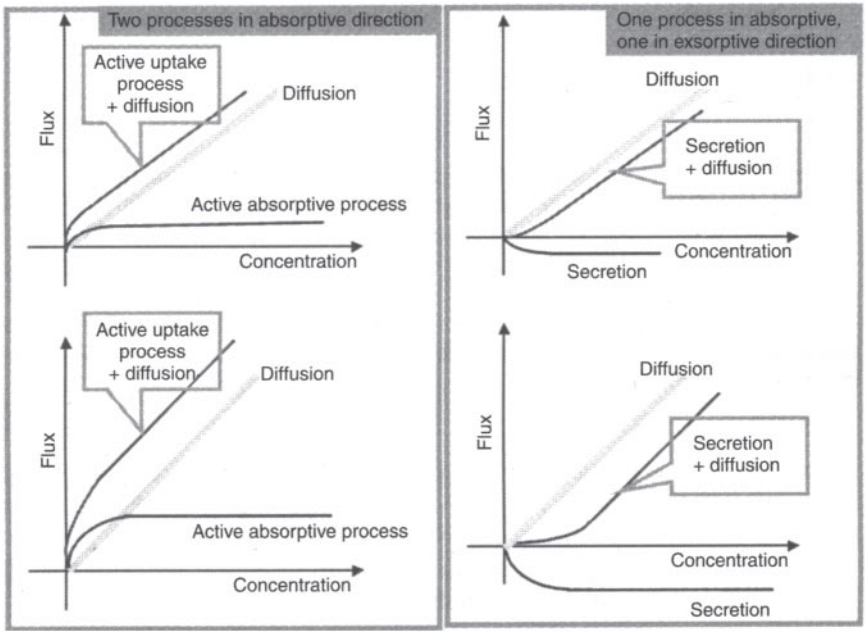

FIGURE 4 Inhibition of transport as interaction mechanism: The relevance of the active inside- or outside-directed transport process depends on the ratio between active and passive processes. When the passive, nonsaturable, and noninhibitable process is dominating, the interaction potential and the potential for nonlinearity are reduced.

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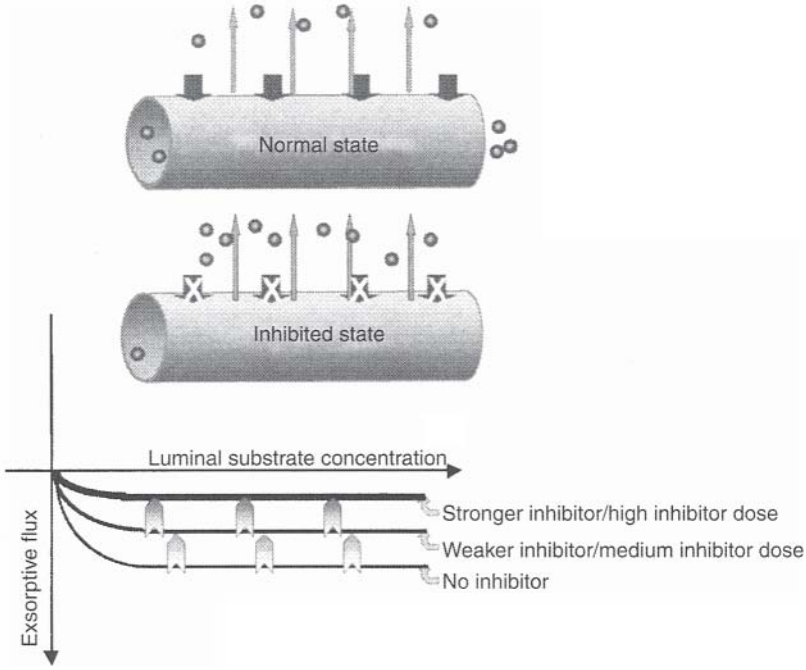

FIGURE 5 General mechanisms for drug-drug and drug-food interactions in the intestine A: Competitive or non-competitive inhibition. Intestinal secretion (exsorption) was chosen as an example for one isolated mechanism of usually many. Like in biotransformation, inside- and outside- directed transport processes may be saturated upon high substrate levels and be inhibited by other compounds with affinity to the respective relevant binding sites. In the case of intestinal secretion, inhibition of the process leads to a higher intestinal permeability and an increase of absorbed fractions.

secretory carrier is increased. For induction of transporters responsible for an absorptive carrier-mediated process, increased apical to basolateral fluxes are to be expected, i.e., bioavailability should be enhanced.

In general, two different scenarios need to be considered with respect to DDIs at the transport or metabolism levels, respectively:

1. A drug **affects** the kinetics of a co-administered compound.
2. A drug **is affected** by a co-administered compound.

Examples illustrating the relevance of the abovementioned mechanisms with respect to DDIs are beginning to emerge. For example, co-administration of substrates of the efflux transporter P-glycoprotein, the product of the multidrug resistance gene (MDR1 in humans), have been

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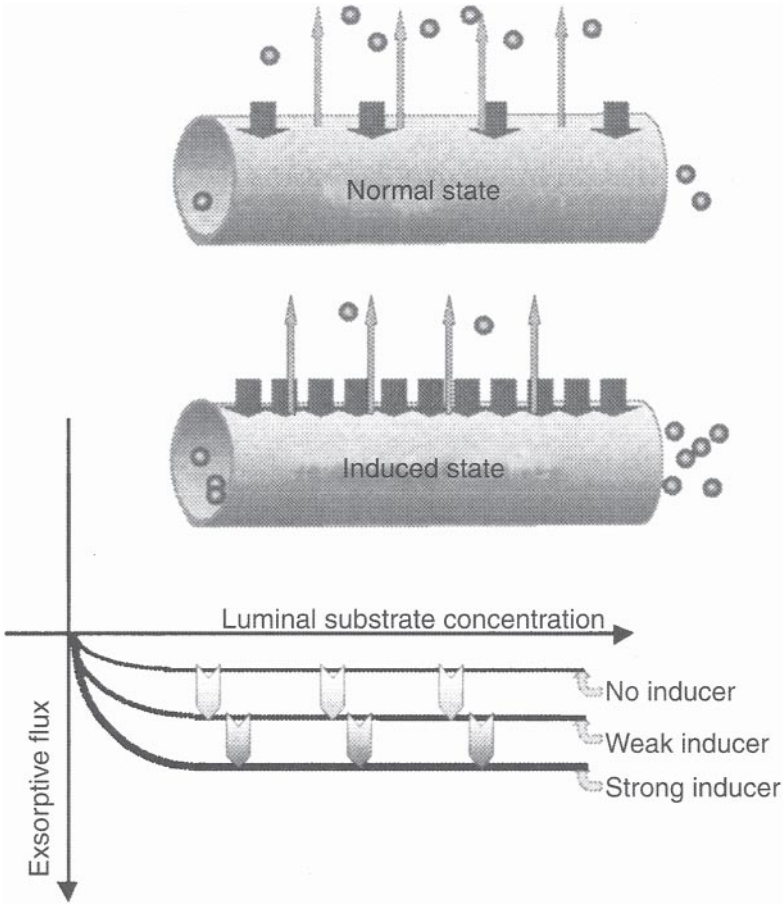

FIGURE 6 General mechanisms for drug-drug and food-drug interactions in the intestine B: Induction. Like in biotransformation, induction of intestinal secretion leads to a decreased intestinal permeability via outside-directed transport and a reduction of the absorbed fraction. Due to induction of exsorption, the relative influence of secretion inhibitors may be higher in the induced state.

well described to result in pharmacokinetic drug-drug interactions. Examples include the interaction of the ß-adrenoceptor antagonist talinolol when administered together with verapamil [31, 32] and erythromycin [33]. From some of these interaction studies it could be seen that the bioavailability of talinolol (increase in rate and extent of absorption) increased when administered together with the co-medication. Also it has been demonstrated by a variety of in vitro, in situ, and in vivo

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techniques, that intestinal secretion of talinolol is saturable and inhibitable by several P-gp modifiers. A much less pronounced effect of the co-medication was observed with respect to changes in talinolol elimination half-life in clinical studies. Nevertheless, preclinical studies have shown [34] that the distribution of talinolol can be significantly modified, when another P-gp substrate or inhibitor is co-administered. Interestingly, also food and food components have recently been described to interact with carriers [35, 36] thus serving as another possibility of explaining peculiar food-drug interactions. Digoxin, another P-gp substrate, is likewise eliminated mainly via excretion of the unchanged moiety. This means, that metabolic drug-drug interactions as the major underlying mechanism of the DDI can be virtually excluded. Digoxin has been described to interact with several P-gp substrates/inhibitors, e.g., talinolol [37, 38], propafenone [39], verapamil [40, 41], quinidine [42], itraconazole [43], ketoconazole [44], clarithromycin [45], rifampin [46], valspodar [47], and atorvastatin [48]. In addition, also for herbal extracts such as extracts of St. John's wort (Hypericum perforatum), which are frequently used as over-the-counter medication, a pharmacokinetic interaction with digoxin has been reported [49]. Interactions of St. John's wort have been reported also in the case of cyclosporine and indinavir, however for the latter two, a contribution of cytochrome P4503A4 to the overall extent of the interaction must be taken into account. Since virtually all of the abovementioned drugs show affinity to *P*-glycoprotein, competition for the binding site or modulation of the function of the multidrug resistance gene product has been made responsible for the observed drug-drug interactions. Since *P*-glycoprotein is widely distributed in absorbing and eliminating epithelia, e.g., small and large intestine, liver and kidneys—among other noneliminating tissues—the increase in digoxin plasma AUC has been attributed to be the result of a decrease in digoxin renal tubular secretion, which is suggested to be mediated by P-glycoprotein, and an increase in digoxin absorption in the GI-tract in the presence of the co-administered drug. Most probably, also altered distribution phenomena have to be taken into account, since it has been shown e.g., in studies with mice, that co-administration of quinidine may increase digoxin brain concentrations in wild-type mice, whereas no increase was reported for the P-glycoprotein deficient mdrla(-/-) knockout mice [50]. Furthermore, a clinically significant interaction at the blood-brain barrier has been described for quinidine, increasing loperamide-induced central effects in humans [51]. Similarly, it is well known that P-glycoprotein is expressed on the brush-border membrane (maternal side) of human placental trophoblast cells and is considered to regulate the transfer of several substances including vinblastine, vincristine, and digoxin from mother to fetus, and to protect the fetus from toxic

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substances [52]. Consequently it may be hypothesized, that co-administration of a P-glycoprotein modulator together with a P-glycoprotein substrate may severely affect the drug concentrations in the fetus. The AUC increase of digoxin upon comedication is generally dependent on the type, dosing regimen, and dose of the co-administered drug. In the case of verapamil, it has been found that digoxin plasma concentrations rose by 60 to 90% [41], whereas with the high affinity modulator valspodar upon multiple dosing, an increase in digoxin AUC of more than 200% has been reported [47]. Since other digitalis glycosides such as digitoxin, α-methyldigoxin, and ß-acetyldigoxin are also substrates of P-glycoprotein [53], it may be hypothesized that similar drugdrug interactions exist for these drugs. Most of the abovementioned drugs are lipophilic and carry at physiological pH—at least to a partial extent—a positive charge. This renders such molecules susceptible to P-glycoproteinmediated transport. On the other hand, transporter-based drug-drug interactions have also been described for a number of organic anions. For example, the loop-diuretic furosemide is subject to polarized transport across renal and intestinal epithelia [54]. The secretion of furosemide can be inhibited with indomethacin. Indomethacin has long been known to inhibit renal clearance of many anionic xenobiotics [55, 56], however, this has not yet been attributed to a single transporter. Instead, the involvement of several transporters is discussed, such as kidney organic anion transporters (OAT), for which *p*-aminohippurate serves as endogeneous ligand [57] and the Multidrug Resistance-Associated Protein (MRP) transporters.

Many drug-drug interactions arise from concurrent administration of drugs which are both substrates and inducers of CYP3A4 and MDR1 expression. Long-term therapy with drugs that induce CYP3A4 and MDR1, for example, increase the systemic clearance of some antileukemic agents, and such therapy has been shown to exert negative effects on survival while increasing cancer relapse [58]. Recent studies have shown, that the steroid and xenobiotic receptor (SXR), a member of the nuclear hormone receptor subfamily which is expressed in the liver and also in the intestine, has a central role in regulating CYP3A4, CYP2C8, and P-glycoprotein transcription via a coordinated mechanism [59, 60]. Thus it is mechanistically understandable, why some Pharmaceuticals such as rifampicin or St. John's wort, induce both the formation of metabolic enzymes as well as the expression of P-glycoprotein. Steroid and xenobiotic receptor thus shows an ability to coordinately regulate multiple xenobiotic clearance pathways and could be regarded as a "steroid and xenobiotic sensor" with a central role of balancing xenobiotic input and elimination as a function of their concentration in the body. Steroid and xenobiotic receptor is activated by a pharmacopoeia of drugs including antibiotics,

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HMG-CoA reductase inhibitors, antiseizure medications, steroids such as glucocorticoids, environmental contaminants such as organochlorine pesticides and polychlorinated biphenyls, and herbal supplements such as St. John's wort. It needs to be investigated, whether screening for SXR affinity is an appropriate measure to distinguish between SXR transparent drugs and potential enzyme inducers. An example on how the safety of a drug can be improved by avoiding SXR affinity is given by the structurally closely related chemotherapeutic agents paclitaxel (Taxol) and docetaxel (Taxotere). Whereas paclitaxel activates SXR and thereby induces its own clearance in a time-dependent manner, docetaxel does not activate SXR or induce drug clearance [60].

In recent years, evidence begins to emerge that variability in pharmacokinetics and drug response may also be in part due to polymorphic variability of drug transporters. For example, for the MDR1 gene (ABCB1), 15 different polymorphisms have been reported, 12 of which did not alter the protein sequence [61]. The mutant C3435T at exon 26 was associated with a lower level of MDR1 expression in enterocyte preparations of the duodenum which was determined by Western blot analysis (P=0.056; n=21). It was also suggested that this exon 26 single nucleotide polymorphism (SNP) correlated with the pharmacokinetics of digoxin, whereby the steady-state C_{max} values of digoxin were 38% higher in volunteers carrying the T/T genotype as compared to the C/C genotype. Another study in 114 healthy volunteers in a Japanese population [62] also performed MDR1 genotyping at exon 26. For the wild-type allele (C/C) 35.1% of the population were found homozygous, 52.6% were heterozygotes (C/T), and 12.3% were homozygous for the mutant allele (T/T). Interestingly, serum digoxin concentrations (AUC_{0-24h} following single dosing) were found to be lower in subjects harboring the mutant T-allele, i.e., C/T and T/T. A satisfactory explanation for the discrepancies between these apparently contradictory findings has yet to be given. In the case of fexofenadine, the C/C mutant resulted in significantly higher plasma concentration-time profiles after peroral administration indicating lower P-gp activity or expression levels in this genotype [63]. An effect of gender or age on the genotype distribution could not be found. However, a sitedirected Ser893 mutation instead of Ala893 in the P-glycoprotein sequence caused by two synonymous SNPs (C1236T in exon 12 and C3435T in exon 26) and a nonsynonymous SNP (G2677T) in exon 21 were found to be linked and occurred in 62% of the European Americans and only in 13% of African Americans, indicating a possible ethnic component in the population distribution. This mutation was significantly correlated with a higher in vitro activity of P-glycoprotein and lower in vivo plasma concentrations of the P-gp substrate fexofenadine in healthy subjects [63]. Ethnic differences in MDR1 polymorphisms were also

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confirmed by a recent study, in which the C3435T mutation was profiled in 1286 research participants from ten different ethnic groups in which significantly higher frequencies of the C/C genotype were found in West Africans and African Americans than in Whites and Japanese populations [64]. An interesting study on the implications of polymorphisms of MDR1 as well as CYP3A4, CYP3A5, CYP2D6, and CYP 2C19 on the pharmacokinetics and dynamics of nelfinavir or efavirenz in HIV-infected patients suggested that plasma concentrations of both antiretroviral drugs decreased in the order of. C/C>C/T>T/T allelic variations in the MDR1 gene [65]. The pharmacodynamic effect of the antiretroviral therapy quantified as an increase in the CD4-cell count was greatest in the T/T genotype, followed by the C/T and C/C genotypes. The finding that P-gp expression in peripheral blood mononuclear cells was lowest in the T/T genotype suggests that the MDR1 polymorphism has significant implications with respect to the admittance of antiretroviral drugs to restricted compartments in vivo. Other hereditary polymorphisms in ABC drug transporters (MRP1, MRP2) are the subject of current investigations [66, 67].

With respect to drug-drug interactions, the consequences of genetic polymorphisms still have to be determined. It may be hypothesized that polymorphisms in transporters which are involved in the ADME cascade of substrates will most likely contribute to the between-subject variability of a drug-drug interaction. The magnitude of the variability will depend on the level of expression of functional transporter protein, the affinity of both drugs to the transporter and the concentration-time profiles of the drugs in the respective organs in which the transporter is expressed. Initial data on the magnitude of transporter induction e.g., by rifampicin also suggest that the magnitude of transporter induction is dependent on the genotype, as has been shown for the MDR1 gene product P-gp [61].

In order to screen for potential DDIs, it may be helpful to study the compound of interest together with certain model compounds. In the literature several compounds appeared, which were found to exhibit affinity to P-glycoprotein and were studied mainly because a DDI was highly probable. This group of compounds is characterized by a certain intermediate hydrophilicity/lipophilicity and intermediate passive permeability. It includes digoxin, fexofenadine, and talinolol (Fig: 7). A highly lipophilic, poorly metabolized, and well-diffusing compound—although a P-gp substrate—may readily pass across membranes and be almost completely absorbed.

With a restriction for talinolol, which is marketed in Europe only, the compounds are well accessible. Moreover, all three have become commercially available as tritium-labeled compound permitting the rapid

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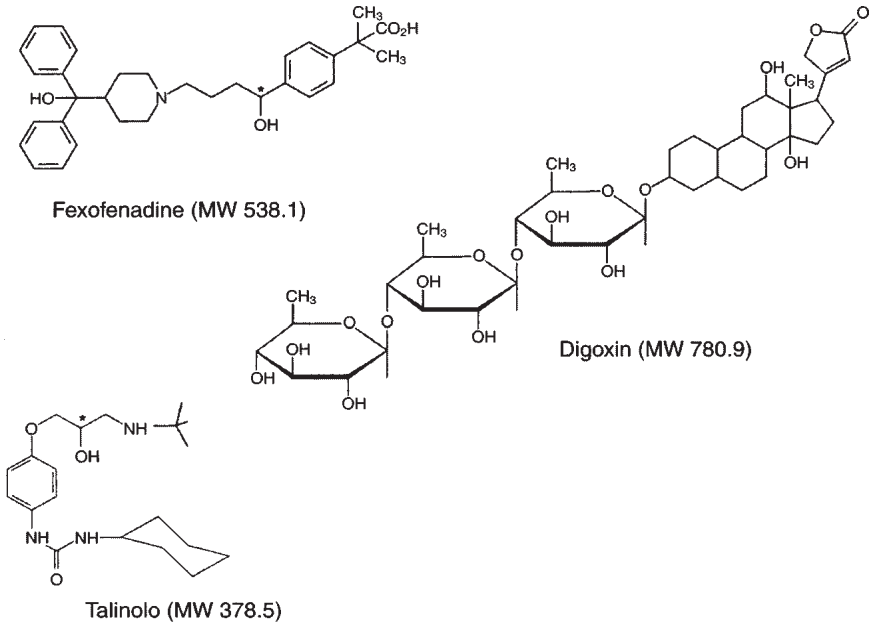

FIGURE 7 Chemical structure of commonly used P-gp substances.

assay of samples from experimental and mechanistic transport studies (in vitro, in situ, in vivo in animals).

Regarding their kinetic behaviour in man, the three drugs have further similarities: They are metabolized to a negligible extent only, i.e., have a high unchanged metabolic clearance, while metabolic substrate loss does not play any significant role. The high therapeutic range observed with the *ß*-adrenoceptor antagonist talinolol and with fexofenadine represents a considerable advantage over digoxin. Selection of an appropriate model compound may be based on the expected side-effects, on the availability of the respective compound and legal considerations.

Digoxin: Potentially because of a favourable passive/active transport ratio, and its traditional availability as a radioactively labeled compound, and in spite of a fairly narrow therapeutic range digoxin has been used as a P-gp model substrate to be influenced by concomitantly administered drugs [50, 68–71]. Its worldwide availability may be an additional reason for its selection.

Talinolol: The use of talinolol as model compound for transport-related processes in, e.g., drug-drug or drug-food interaction studies as previously

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proposed [36] appears reasonable because of its mainly unchanged renal and biliary clearance, the low protein binding (approximately 25%), and the sensitivity of its kinetics for changes in P-glycoprotein expression, but also to transporter function (inhibition by P-gp modulators). Only to a small extent these advantages are neutralized by a potential for affinities to other transporters: ^{3}H-Tetraethylammonium uptake studies in LLC-PK$_1$ cells revealed an inhibiting effect of talinolol on TEA uptake, which indicates an additional interaction with the OCT [72]. Furthermore, there is evidence from in vivo studies with MRP2 deficient rats that MRP2 also contributes to talinolol disposition to some extent.

It may be considered to use the distomer instead of the racemate, since the eudismic ratio for talinolol is approximately 40 and no significant Pglycoprotein affinity difference was detected for the enantiomers.

Fexofenadine: Fexofenadine, a nonsedating antihistamine and metabolite of terfenadine does not—like talinolol—undergo significant metabolic biotransformation. Employing different cell lines, evidence was found that uptake and efflux transporters are involved in fexofenadine absorption and disposition [73, 74]. Among various transport systems investigated, the human organic anion transporting polypeptide (OATP) and rat organic anion transporting polypeptides 1 and 2 (Oatpl and Oatp2) were identified to mediate [^{14}C]-fexofenadine cellular uptake, while P-gp was identified as fexofenadine efflux transporter, using the LLC-PK$_1$ cell, the polarized epithelial cell line lacking P-gp, and the P-gp overexpressing derivative cell line L-MDR1. Studies in P-gp knock-out mice confirmed the relevance of this transporter for fexofenadine disposition in a similar way as demonstrated for talinolol, for which a high relevance of P-gp for absorption and disposition was detected [75].

Interactions as a Result of Alterations in Plasma Protein Binding

Competition for protein binding sites is likely when two drugs are highly bound to plasma proteins. The displacement of a drug from its binding site at the protein is frequently followed by an increase in its unbound drug concentration. Since it is the unbound drug that is pharmacologically active, this increase in "free" drug tends to increase the pharmacodynamic effect of the displaced drug. The conditions favoring displacement have been outlined previously [76]. In addition to the type and concentration of the respective binding protein (600 uM for albumin and 9–23 uM for α1-acid glycoprotein) the plasma concentrations of the drug and the displacer and their affinities to the binding sites are of relevance. Displacement of drugs that bind to α1-acid glycoprotein is more likely to occur as a result of the lower blood concentrations of this protein as compared to albumin. An initial increase in the unbound plasma concentration of a low extraction

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drug (restrictively cleared drug) may however be readily compensated by an increase in its clearance, and an additional buffering effect by an increase in its volume of distribution. Thus, although total drug plasma concentrations may be diminished in an interaction situation, the unbound concentrations of the drug may remain constant and no dosage adjustment needs to be made. An example is the displacement of phenytoin by valproic acid. Coadministration of valproic acid to phenytoin has been reported to decrease total steady-state plasma phenytoin concentrations in a dose-dependent manner [77]. In accordance with the theory, unbound concentrations of phenytoin remained constant in that study. On the other hand, the theory of plasma protein binding displacement interactions being the common cause of clinically significant interactions has been questioned [78]. In the case of valproic acid and phenytoin, additional mechanisms are likely to be the major ones responsible for the exaggerated effect observed clinically [19]. In addition to the displacement from plasma protein binding sites, going along with an increased distribution of the drug throughout the rest of the body and concomitant enhancement of the systemic clearance of total drug, an inhibition of phenytoin metabolism by valproic acid and thereby an increase in the concentration of free drug in the serum has been described [80]. Likewise additional mechanisms are likely to be involved in the causes of drug-drug interactions with clinically observed exaggerated effects, e.g., the interaction of warfarin with phenylbutazone leading to marked increases in prothrombin times and the interaction of sulphonamides with tolbutamide resulting in a sustained increase in hypoglycaemic effect, as well as the toxic interaction between acetazolamide and salicylate [81]. In all cases, a reduction of the clearance of free drug has been made responsible for the accumulation of the displaced drug, thus making the hypothesis of a drug-drug interaction purely driven by plasma protein displacement unlikely. For high clearance drugs (unrestrictively cleared, flow-limited) administered intravenously, increased free concentrations following displacement will not be adequately compensated by increased clearance, as both free and bound drugs are already available for elimination by the clearing organ and clearance will be most sensitive to changes in organ blood flow rate. Thus the increased free-drug concentrations will possibly result in an enhanced response. Examples for drugs, where protein-binding displacement may be clinically significant include lidocaine, alfenanil, buprenorphine, fentanyl, hydralazine, midazolam, and verapamil [82]. For nonrestrictively cleared drugs (hepatic clearance) which are given perorally, the increase in the free fraction may cause a slight increase in hepatic extraction and a decrease in bioavailability, which will lead to a reduction in steady-state concentrations (C_{ss}). The combined effect of an increase in fu and a decrease in Css, however, means that unbound steady-state concentrations of the drug being displaced will be

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largely unaltered compared with the predisplacement value. There are very few perorally administered drugs that exhibit the properties of extensive plasma protein binding and high hepatic first-pass extraction, for example propranolol, imipramine, and desipramine. Those, however, tend to have a relatively wide therapeutic margin.

Physicochemical Interactions and Interactions based on Changes in Local pH and Ionization State of Molecules, Respectively

A few drugs have structures that readily form chelate complexes with divalent or trivalent cations such as aluminium, magnesium, iron, or calcium. The complexed drugs are not absorbed across the intestine and hence their plasma concentrations may be subtherapeutic. Examples include quinolone antibiotics (e.g., ciprofloxacin) and tetracyclines which are markedly less absorbed when administered together with magnesium-aluminium antacids. Other cations, such as calcium, iron, and probably zinc, appear to interact in a similar manner. Cholestyramine is a basic anion-exchange resin used in the treatment of hypercholesterolemia. The hydrophilic but water insoluble powder is not absorbed in the GI tract, however, it can adsorb bile acids and a number of drugs (e.g., digitalis glycosides, coumarin, diuretics, quinidine, thyroxine, propranolol, and some antibiotics). As a safety precaution it has been recommended to discontinue resin administration for short-term courses of antibiotics, corticosteroids, pre- and postoperative medications, rather than risking the possibility of the action of the drugs being diminished or abolished by the interaction with the resin. Malabsorption of lipophilic drugs has also been observed when the drugs were administered together with nondigestible oils. Here, it is likely, that the drugs will dissolve in the oil and thus may not be available for absorption [83, 84]. In some instances, nondigestible oils have been used for enhancing the intestinal elimination of toxicants [85, 86]. Other studies have shown though, that upon proper spacing of the intake of nonabsorbable fat replacements and lipophilic drugs, an interaction can be avoided [87, 88]. Activated charcoal is another drug with several potential interactions based on surface adsorption. This is due to its large surface area of approximately 1000 m^2g^{-1}, which however varies from one charcoal preparation to another. Some of the documented interactions include anticonvulsants and oral emetics as well as oral antidotes for acetaminophen poisoning such as methionine [89]. Drug-drug interactions based on changes in the ionization state of molecules are of particular relevance for processes and compartments, in which significant changes in the local pH occur. Such compartments are the kidneys and the stomach. The pH in the stomach may vary considerably, also as a function of

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co-administered drugs. For example, the median pH in a control group of gynecologic out-patients increased from 2.2 to 5.7 following treatment with 400 mg of cimetidine [90]. As a consequence, the dissolution and absorption of basic drugs with low water solubility, such as ketoconazole, is diminished in cases of lowered gastric acidity [91]. Similar observations have been made for itraconazole.

Changes in urinary pH may alter the tubular reabsorption of drugs with pka values in the physiological range. Thus weak acids such as salicylic acid, barbiturates, and sulfonamides show higher renal clearance at alkaline urine pH. On the other hand, weak bases, such as amphetamine, antihistamines, imipramine, and meperidine are preferentially cleared at acidic urinary pH values (approximately 5).

Drug-drug Interactions based on Pharmacodynamic-Pharmacokinetic and Pharmacodynamic Mechanisms

Pharmacodynamic-pharmacokinetic drug-drug interactions originate from situations, where a pharmacological effect of a particular drug can modify the pharmacokinetics of a second drug. For example, a compound which affects the gastrointestinal motility may influence the rate and extent of absorption of another co-administered drug by altering gastric emptying times and passage times across the small intestine. Thus the absorption of paracetamol can be delayed with concurrent administration of propantheline, a muscarinic receptor antagonist and with opiate-type analgesics. Metoclopramide and other prokinetic agents however, increase motility and transit of material in the gastrointestinal tract. The question as to whether the extent of drug absorption of a particular compound is modified by a prokinetic agent is frequently dependent on the intestinal permeability of the drug. For compounds with high permeability, the extent of drug absorption remains unchanged, since the residence times in the absorbing segments are more than sufficient to ensure complete absorption. Thus, even an increase in the gastrointestinal transit times will manifest in a change in rate but not extent of drug absorption. Another example for a pharmacodynamic-pharmacokinetic interaction is the interaction between compounds which modify the blood flow through the major clearing organs and high-clearance drugs. Propranolol, for example, by reduction of the cardiac output, diminishes the liver blood flow and reduces its own clearance and the clearance of lidocaine and bupivacaine [92, 93]. Similar interactions due to modification of blood flow in target tissues have been observed with anaesthetic agents. For example, volatile anaesthetics have been shown to delay the intramuscular absorption of ketamine in addition to diminishing the volume of distribution and clearance of a number of high-clearance compounds [94].

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REGULATORY ASPECTS OF DRUG-DRUG INTERACTIONS

FDA Guidance on in vivo Metabolic Drug Interactions Studies

In November of 1999, the FDA issued a guidance on the study design, data analysis, and recommendations for dosing and labeling of in vivo metabolic drug interaction studies [95]. The basic concepts that are behind the recommendations in this guidance are as follows:

1. An understanding of the metabolic fate of a drug and the contribution of metabolism to the overall elimination of the drug is essential in the assessment of its safety and efficacy profile.
2. It is important to elucidate whether the investigational drug affects the metabolism of currently marketed drugs and conversely whether the metabolism of the investigational drug is also affected by currently available drugs.
3. Sometimes even though a drug might not be metabolized, it still can be a potent inhibitor of a certain metabolic pathway. Thus it is important to elucidate its effect on the metabolism of currently marketed drugs metabolized by the inhibited enzymes.
4. The clinical importance of a drug interaction sometimes depends on the genetic polymorphism (whether a patient is considered a slow or fast metabolizer) of the individual. Moreover, other covariates such as age, race, and gender can be of prime importance in the clinical outcome of the interaction.

Study Design Considerations

Dosing Regimens. One of the major considerations in designing a drug-drug interaction study is whether to dose the substrate (S) or the interacting drug (I) as single dose or chronically (multiple dose).

The selection of the dosing regimens will depend on

a. Whether the S or I is dosed acutely or chronically in the clinical setting
b. Safety considerations including whether the drugs are considered narrow therapeutic index or not
c. The pharmacokinetic and pharmacodynamic characteristics of the S and I
d. The need to assess induction or inhibition.

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A recent survey of all approved new molecular entities, approved between 1992–1997, showed that the preferred dosing regimen was to dose both I and S to steady state (47% of all studies) while in 30% of the cases one of the drugs was dosed to steady state [96]. The use of such designs is a reflection of the clinical use of these drugs and the fact that for inducers and some inihibtors it might take several days to see the full extent of the interaction. As an illustration to this point, an interaction study between alfentanyl and erythromycin did not show any interaction on the clearance of alfentanyl. However, after a seven-day course of 500 mg erythromycin twice daily, there was a 25% decrease in alfentanyl clearance and a 60% increase in the alfentanyl half-life [97]. Another complicating factor in the ability to extrapolate the single dose findings to steady-state situations is the potential for certain inhibitors to also act as inducers when given on a long-term basis. One such drug is the protease inhibitor ritonavir.

On the other hand, the vast majority of absorption-based drug interaction studies with drugs such as antacids or drugs that affect gastric motility use a single single-dose study design since with this design one can determine whether the bioavailability of the S is affected.

Study Population

The vast majority of drug-drug interaction studies employ healthy volunteers as the study population since it is assumed that the findings obtained from such a population can easily be extrapolated to the patient population for which the drug is intended. However, in certain instances where safety considerations precludes the use of healthy volunteers, or in situations where the pharmacodynamic endpoints to be measured in the study cannot be easily extrapolated to the patient population, one is forced to recruit from the general patient population. In either case, performance of genotype or phenotype determinations to identify genetically determined metabolic polymorphisms is often important in evaluating enzymes such as CYP2D6 or CYP2C19.

Statistical Design Considerations

The number of subjects to be enrolled in the study depends on the magnitude of the effect to be detected that is considered to be clinically relevant, the inter and intrasubject variability in the PK measurements and any other factors that might affect the outcome of the study.

The most common statistical design for pharmacokinetic drug interactions is the crossover design accounting for half of all the studies submitted to the Agency from 1987 to 1997. More recently an increased reliance on a fixed sequence design (where a subject receives a drug for a

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fixed period and the second drug is introduced at a certain time in the dosing period). Such a design is considered to be a variation of the crossover design. A parallel design is most useful in situations where one of the studied drugs or its metabolites have a long half-life.

According to the FDA guidance, the results of the drug-drug interaction studies should be reported as 90% confidence intervals about the geometric mean ratio of the observed PK measure with and without the interacting drug. Confidence intervals will provide an estimate of the distribution of the observed systemic exposure with and without the interacting drug and thus conveying a probability of the magnitude of the interaction. On the other hand, tests of significance are not appropriate for such studies due to the fact that clinically insignificant exposure differences can achieve statistical significance without having to recommend dosing adjustments or contraindications.

Moreover, the FDA guidance recommends that in a drug-drug interaction study, the sponsor of the investigational drug should be able to provide specific dosing recommendations based on what is known about the PK/PD relationship or the dose-response relationship. Unfortunately such information is not always available especially for drugs that are already on the market.

If the sponsor intends to make a specific claim in the package insert that no drug interaction is present, the sponsor should be able to recommend specific "no effect boundaries" or clinical equivalence intervals defined as the interval within which the change in a systemic exposure measure is considered to be clinically not relevant.

The guidance recommends three approaches in defining these no effect boundaries:

Approach 1:

The no effect boundaries are based on population average dose-response or exposure-response relationships and any other available information for the drug under study. If the 90% confidence interval for the systemic exposure measure falls within the no effect boundary, then it may be concluded that no clinically significant drug-drug interaction is present.

Approach 2:

The no effect boundary may also be based on the concept that a drug-drug interaction study addresses the question of switchability between the substrate given alone and in combination with an interacting drug. In this case, a sponsor may wish to use an individual equivalence criterion to allow for scaling of the no effect boundary.

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Approach 3:

In the absence of no effect boundaries as defined in Approach 1 or 2, a sponsor may use a default no effect boundary of 80–125% for both the investigational drug and the approved drugs used in the study. When the 90% confidence intervals for systemic exposure fall entirely within the equivalence range, the Agency in most cases will conclude that clinically significant interaction is present.

It is to note that Approach 3 does not necessary imply that the sponsor needs to always power the study in a way that the 90% confidence interval for the ratio of pharmacokinetic measurements falls entirely within the no effect boundary resulting in an increased number of subjects for each study.

Choice of Substrate and Interacting Drugs

Substrates for an Investigational Drug. If the investigational drug is an inhibitor of a specific enzyme system, the substrate to be selected as the interacting drug should be one whose pharmacokinetics is markedly altered by the inhibitor. The guidance includes several examples of substrates such as midazolam, buspirone, felodipine, simvastatin or lovastatin for CYP3A4, theophylline for CYP1A2, S-warfarin for CYP2C9, and desipramine for CYP2D6. If the initial study was found to be positive, further studies of other substrates might be recommended based on the likelihood of coadministration. If the initial study was found to be negative with the most sensitive substrate, then it is safe to assume that the less sensitive substrates will also not be affected.

Investigational Drug as a Substrate. The testing of the investigational drug as a substrate will depend on the results of the in vitro metabolic studies identifying the enzyme systems that metabolize the drug. If for example the investigational drug is shown to be metabolized by CYP3A4 to a great extent, the choice of inhibitor and inducer could be ketoconazole or rifampin since both of these drugs are known for their substantial effect on this pathway. If the results of such a study are deemed negative, then the absence of an interaction for this metabolic pathway could be claimed by the sponsor. However, if the study found a clinically significant interaction for this metabolic pathway, and the sponsor would like to claim a lack of interaction with a less potent inhibitor/inducer then more studies would be recommended to substantiate the specific claims with regard to the less potent interactants.

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Route of Administration

In general, it is recommended that both the substrate and interacting drug be administered in the same way these drugs are used (or going to be used clinically). However, if multiple routes of administration are possible, it might be necessary in some cases to investigate the possibility of drug interactions with the different routes of administration. This is particularly true for drugs that undergo gut wall metabolism whereby the amount of metabolism will differ between the oral and intravenous routes. Therefore it is thought that the differences in exposure that result from a drug interaction will be different depending on the route of administration (viagra interaction with erythromycin), which will consequently result in different dosing adjustment recommendations, then in such cases one is better off obtaining the true magnitude of interaction for the different routes of administration.

Dose Selection

Unless there are overriding safety concerns it is recommended to use the highest possible dose for both the substrate and the interacting drug and the shortest dosing interval. This will maximize the probability of finding an interaction and will also shed light on the possible maximal magnitude of the interaction and the worst case scenario in the change in exposure that will result in a clinically significant interaction such as dosing adjustment or even a recommendation to contraindicate the co-administration of the two drugs.

Labeling

The FDA guidance recommends the inclusion of both positive and relevant negative findings of the results of the in vivo drug interaction studies in the "Clinical Pharmacology" section under "drug-drug interactions." If the results of the study indicate a potentially clinically significant interaction or the lack of an important interaction that might have been expected, in addition to mentioning it in the "Clinical Pharmacology" section, a more detailed description of the study and its results should be included in the "Precautions" section of the label with advice on how to adjust the dosage in the "Warnings/Precautions," "Dosage and Administration," and "Contraindications" sections of the label. The FDA guidance allows the extrapolation of the results of a drug-drug interaction study with a certain substrate or inhibitor to other substrates or inhibitors/inducers not specifically tested thus allowing for a class label based on the results of the study with a drug that is considered a prototype. For example, if an

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investigational drug is a potent CYP3A4 inhibitor, not all substrates of this enzymes need to be tested to warn against an interaction with this drug.

The following are examples of appropriate labeling language recommended by the FDA guidance:

Drug-Drug Interactions, Clinical Pharmacology

X In vivo metabolic drug-drug interaction studies indicate little or no pharmacokinetic effect:

Data from a drug-drug interaction study involving (drug) and (probe drug) in____ patients/healthy individuals indicate that the PK disposition of (probe drug) is not altered when the drugs are co-administered. This indicates that (drug) does not inhibit CYP3A4 and will not alter the metabolism of drugs metabolized by this enzyme.

X In vivo metabolic drug-drug interaction studies indicate a clinically significant pharmacokinetic interaction:

The effect of (drug) on the pharmacokinetics of (probe drug) has been studied in____ patients/healthy subjects. The Cmax, AUC, half-life, and clearances of (probe drug) increased/decreased by ____% (90% Confidence Interval: ____ to ____ %) in the presence of (drug). This indicates that (drug) can inhibit the metabolism of drugs metabolized by CYP3A4 and can increase blood concentrations of such drugs. (See Precautions, Warnings, Dosage and Administration, or Contraindications sections.)

Precautions and/or Warnings

X An interacting drug causes increased concentrations of the substrate but the administration of both drugs may continue with appropriate dosage adjustment. Results of the studies are described in *Clinical Pharmacology, Drug-Drug Interactions, Precautions* and/or *Warnings* and may state:

Drug____/class of drug causes significant increases in concentrations of ____ when co-administered, so that dose of ____ must be adjusted *(see Dosage and Administration)*. If there is an important interaction, information for patients should point this out also.

X An interacting drug causes increased risk because of increased concentrations of the substrate and the interacting drug should not be used with the substrate. After describing the interaction in the *Clinical Pharmacology* section, there should be a *Contraindications* section and possibly a boxed warning if the risk is serious.

Drug____/class of drug can cause significant increases in concentrations of drug____ when co-administered. The two drugs should not be used together.

Dosage and Administration

X An interacting drug causes increased risk because of increased concentrations of the substrate, but the administration for both drugs may continue with suitable monitoring: *Drug____/class of drug leads to*

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significant increases in blood concentrations of ____ by____%. The dose of ____ should be decreased by ____% when the patient is also taking ____. Patients should be closely monitored when taking both drugs.

Contraindications

X An interacting drug causes increased risk because of increased concentrations of the substrate and should not be co-administered:

Drug____/class of drug leads to significant increases in blood concentrations of ____, with potentially serious adverse events. Administration of ____ to patients on drug____/class of drug is contraindicated.

European Guidance on the Investigation of Drug Interactions

The European Agency for the Evaluation of Medicinal Products issued in December 1997 a note for guidance on the investigation of drug interactions [98]. This guidance took effect in June 1998. Unlike the FDA guidance which only dealt with the in vivo metabolic aspects of drug interactions, the European guidance covered both pharmacodynamic and pharmacokinetic drug interactions (absorption, distribution, and elimination both at the renal excretion and hepatic/biliary levels as well as changes in blood flow).

The European guidance makes certain recommendations that are either not covered or sligthly differ from the FDA recommendations. These recommendations are as follows:

A. The need for a pharmacodynamic interaction study should be determined on a case by case basis taking into account the following points:

1. When the drugs likely to be co-administered have similar mechanisms of action or potentially similar interaction mechanisms.
2. When drugs likely to be co-administered have similar or opposing pharmacodynamic effects.

B. In vitro studies may be helpful in investigating the transport mechanism and whether a drug is a substrate or an inhibitor of P-glycoprotein. However, the guidance recommends that potential interactions at this level be confirmed by well-designed in vivo studies since current in vitro studies have shown to be of limited value in predicting the magnitude of the interaction.

C. Displacement interaction studies should be performed when the investigated drug:

- Has nonlinear protein binding.
- The volume of distribution is small.
- Has a narrow therapeutic index.

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- Is highly bound (>95%) to plasma proteins at therapeutic concentrations.
- Occupies most of the binding sites (such as when the plasma therapeutic concentrations at the highest recommended dose exceed the plasma binding capacity).
- When the investigated drug is administered intravenously and possesses a high metabolic extraction ratio.
- Displacement studies should probably be done in vivo, since the metabolites may also be involved in the interaction. If the studies are performed in vitro, then the possible contribution of the metabolites should also be considered.

D. In general, the guidance recommends conducting an in vitro or in vivo metabolic interaction studies for metabolic pathways responsible for 30% or more of the total clearance. However, if toxic/active metabolites are formed by minor metabolic pathways, the effect of co-administered inhibitors or inducers of these pathways should also be investigated.

E. Subjects participating in metabolic in vivo interaction studies should be appropriately genotyped and/or phenotyped if any of the active enzymes mediating the metabolism are polymorphically distributed in the population.

F. For inducers or inhibitors, steady-state conditions should be achieved whenever possible. Approved therapeutic dose regimens should be used in these studies.

Canadian Guidance on Drug-Drug Interaction Studies

The Therapeutic Products Program of the Canadian Health Agency issued a guidance document in May of 2001 entitled "Drug-Drug/interactions: Studies In Vitro and In Vivo" [99]. This guidance as the title indicates covers both in vitro and in vivo studies. Since the recommendations that are given in this document do not differ from the recommendations of the U.S. FDA guidances on this topic, this guidance will not be discussed in detail in this chapter.

However of interest is this guidance recommendation on how to report the findings of these studies in the product monograph.

According to this guidance all documented and anticipated drug interactions should be included in the "Drug Interactions" subsection of the "Precautions" section with appropriate cross references to other sections of the label. Drug interactions should be presented as contraindications if they have the capacity to be life-threatening, cause permanent damage, or elicit other reactions that would prohibit concomitant administration. Interactions having the potential to cause serious or severe consequences that are

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reversible or not life-threatening should normally be included in the "Warning" section together with recommendations for appropriate risk management measures. Drug interactions of unknown clinical significance or resulting in adverse effects that are merely bothersome can generally be adequately dealt with in the "Drug Interactions" subsection of the "Precautions" section.

In addition when describing the results of in vivo clinical drug interaction studies, the monograph should indicate the number of subjects studied, and whether they were healthy volunteers or patients. The dose and duration of treatment should also be described.

Drug interactions identified through population pharmacokinetic approaches, clinical trial case reports, or spontaneous postmarketing adverse event reporting should be identified as such. The guidance recommends that in cases where sufficient information is available comments on the mechanism of the interaction, the clinical manifestations, as well as actions to prevent or respond to an interaction should be provided.

As for class labeling, the guidance recommends that manufacturers not wanting a class labeling with regard to drug interactions should submit data showing that the possibility of such interactions with their products has been adequately investigated and dismissed.

ROLE OF POPULATION PHARMACOKINETICS IN THE STUDY OF DRUG INTERACTIONS

Collecting sparse sampling during the larger phase III clinical trials can help identify both the intrinsic and extrinsic factors that might affect exposure to a drug. Thus using such a screening approach might be valuable in detecting unsuspected drug-drug interactions especially in patients exhibiting a higher incidence of side effects. Both the U.S. FDA guidance and the Canadian guidance state that a well-executed population analysis can provide further evidence of the absence of a drug interaction when in vitro data suggest the lack of one. However, on the other hand both guidances agree that the sparse sampling approach to detect a drug interaction is not yet well established and that it is unlikely that one will be able to rule out an interaction that is strongly suggested by information that is obtained from in vitro or in vivo studies specifically designed to detect an interaction. This is due to the presence of confounding variables that are not controlled in the study that reduce the power to detect an interaction. The major advantage of such an approach is that the study is conducted in the target patient population and thus clinical inferences on the magnitude of the interaction as well as

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dosing recommendations are easier made from the results obtained. Another advantage of such an approach is that it does not expose healthy volunteers to unnecessary side effects of the drug. However, these studies are considered to be much more difficult to perform and believed by some to be more costly [100, 101].

CONCLUSION

There is an increased awareness both by the regulatory authorities and by drug sponsors on the importance of the elucidation of the potential for drug interactions of a new molecular entity. Establishing the drug interaction profiles of a new drug and providing proper information on dosing recommendations when certain drugs are given together is an important risk management tool and will go a long way in avoiding unwanted adverse events.

A well-designed program that takes into account the available in vitro technologies, the right in vivo studies, the appropriate model compounds and a population screen during the phase III trials will not only provide the necessary information that is required by regulatory agencies but will also provide guidance to the prescriber and patient on the appropriate dosing recommendations when multiple drugs are co-administered [102].

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Assessing the Effect of Disease State on the Pharmacokinetics of the Drug

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INTRODUCTION

Efficacy and safety of a new medicinal product are established in phase III trials conducted in a selected group of patients. In fact, with the aim to reduce the variability, there have been an increasing number of inclusion and exclusion criteria imposed in the phase III studies submitted to the Medical Products Agency in Sweden over the last 10 years. However, when approved, the product is often used in a wider group of patients. To compensate for this discrepancy the pharmaceutical industry and regulators use pharmacokinetic data, together with studies in animals, to identify subgroups of patients where the exposure is changed to an extent that they should not be treated with the medicinal product, or the dose needs to be adjusted. The aim of this chapter is to discuss disease states that may influence the pharmacokinetics of a medicinal product. References are made

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to a number of regulatory guidelines. It is, however, important that these are considered to be guidelines and nothing more than guidelines. Each new drug has its own characteristics and should be developed according to current scientific standards.

METHODOLOGICAL ASPECTS

The impact of disease on the pharmacokinetics can be evaluated either in specific studies or by population pharmacokinetic analysis of data from phase II–III studies. However, the many inclusion and exclusion criteria in today's phase III studies may limit the possibility to use a population approach. Such an approach requires that a sufficiently large number of patients with different degrees of dysfunction are included in the study, otherwise the results are of limited value. When sufficient data are available, results from population analysis alone are fully sufficient for labeling purposes.

When designing or assessing a study in a specific patient population, there are often a number of pharmacokinetic issues that need to be considered, including:

- Relationship between concentration and response (both desirable and undesirable effects) i.e., how much can the concentration change without influencing the efficacy or safety of the drug.
- Given the intended therapeutic use of the drug, what is the major concern: concentration-dependent side effects or lack of efficacy?
- Variability in the population (are outliers cause for concern?)
- Is it reasonable to assume that the pharmacodynamics is the same in different subpopulations?

Obviously, the answers to the questions above and the selected study design should be based on the pharmacokinetic/pharmacodynamic characteristics of the drug. The additional issues that need to be considered include:

- Are there any nonlinear properties that would justify steady-state studies? A multiple-dose study is desirable when the drug or an active/toxic metabolite is known to exhibit nonlinear or time-dependent pharmacokinetics. Otherwise a single-dose study is sufficient.
- Dose selection. In single-dose studies, a dose within the therapeutic dosage range should be used. For multiple-dose studies, lower or less frequent dosing may be needed to avoid unsafe accumulation of drug and/or metabolites.

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- Which pharmacokinetic parameters are of greatest concern? Extent of bioavailability (F) and clearance (CL) are often most important, usually measured as AUG. When appropriate, emphasis should also be given to rate of absorption or other "secondary" parameters such as C_{max}.
- Should only the parent compound be measured or should also the active/toxic metabolites be determined? If the metabolites are active or toxic, the impact of disease states on these metabolites should be evaluated. Evaluation of inactive metabolites should be considered when appropriate.
- Should the pharmacokinetics be based on total or unbound drug? For example, when plasma protein binding may be altered, the pharmacokinetics should be described and analyzed with respect to the unbound concentrations of the drug and active metabolites in addition to total concentration, unless the drug or metabolites exhibit relatively low extent of plasma protein binding.

In addition to selecting which trials should be conducted, the sponsor must also decide when to perform the studies. If available, information on influence of disease on the pharmacokinetics of the drug could be of value when designing the phase III programme. On the other hand, there may be financial as well as ethical reasons to perform these studies late in phase III or even after a regulatory approval of the medicinal product. In the latter situation, a specific subgroup may be contraindicated pending availability of this information.

TARGET POPULATION

Introduction

Several factors may induce a difference in pharmacokinetic parameters between volunteers and target population, such as disease-related factors and demographic factors (e.g., age, gender, and weight). The rate and extent of absorption, the extent of distribution and/or the elimination rate could be altered as a consequence of a disease. The disease is a large source of variability in drug response between patients and the variability can, at least in part, be attributed to the pharmacokinetics.

Disease-related pharmacokinetic differences between target patients and volunteers can largely be explained by functional disturbances of the eliminating organs, liver and kidney separately discussed later in this chapter. But, even when renal and hepatic elimination has been accounted for, pharmacokinetic differences between populations may persist. For a number of disease states, an effect on the pharmacokinetics is not expected. Examples of such conditions are pain (at least mild to moderate), mild infections, skin disorders, psychiatric

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diseases. Others are more likely to induce a pharmacokinetic change. These include cardiovascular disorders with effects on perfusion rate, endocrine dysfunction as diabetes causing reduced renal function and altered protein binding and severe respiratory disorders that may give hypoxaemia and disturbance in the acid-base balance. Ultimately, whether or not a significant disease-related change will appear depends on the pharmacokinetic characteristics of the drug, e.g., elimination pathways, high- or low-hepatic extraction, degree of protein binding. So far, there is no specific guideline addressing pharmacokinetic differences due to disease factors.

Studies in Healthy Volunteers and Patients

The pharmacokinetic characteristics of a drug are usually evaluated in early studies conducted in, if ethical, healthy volunteers (HV) under well-defined and controlled conditions. Multiple-dose studies are conducted either in a selected patient population suffering from the disease for which the drug is considered to be indicated and/or in HV. In later studies, the pharmacokinetics in the target population is evaluated using various approaches, such as gathering full pharmacokinetic profiles in limited numbers of patients or obtaining few steady-state concentrations measurements e.g., sparse sampling [2]. From these results, a relation between the grade of illness and the impact on pharmacokinetic parameters could be established. However, comparisons between volunteers and patients are usually confounded by demographic variables, for instance age or weight, which also have a potential to affect the pharmacokinetics, and hence such divergence has to be recognized and assessed.

The European guideline, Pharmacokinetic Studies in Man [1], states that "Studies should be conducted in patients suffering from the disease for which the drug is claimed to be indicated. If feasible, the relation between dose, plasma concentration, and effect should be studied. Particularly, it should be established that the pharmacokinetic behaviour of the drug in patients corresponds to that in healthy volunteers. The full range of pharmacokinetic studies needs only be repeated in patients if studies indicate that the pharmacokinetics in this group differ from those in healthy volunteers." The last sentence leaves the subject open for interpretations, since the word "differ" has not and cannot be defined quantitatively. If an important difference is detected it is still questionable whether all studies have to be repeated. Instead, the number of studies necessary should be judged on a case-by-case basis depending on the degree and type of difference and also the general characteristics of the drug. If there is reason to believe that certain physiological or pathological factors, such as certain functional or anatomical disorders of the gastrointestinal tract, might substantially alter absorption, separate pharmacokinetic studies in suitable

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volunteers or patients could be performed. Information about pharmacokinetic differences between healthy and patient populations should be included in the labeling of the drug.

For drugs displaying marked pharmacokinetic divergence between populations, predictions based on HV data might not adequately enough characterize the target patients. The following issues need to be considered.

- Further studies to evaluate the pharmacokinetics in special populations, e.g., renal and hepatic impairment, are conducted in individuals not necessarily suffering from the target disease, and hence reducing the predictability. Moreover, healthy volunteers are often chosen as the control group, whereas the target population would be a more appropriate control group, given a difference in pharmacokinetics.
- Conventional interaction studies are often conducted in healthy volunteers and the results cannot always be extrapolated to the target population. For instance, there might be disease or demographic-related factors affecting the drug absorption differently in the target population, increasing or decreasing an interaction on bioavailability. Furthermore, the patient might use concomitant therapy that is not taken into account in the volunteer study.
- Usually, the interindividual variability in pharmacokinetic parameters is lower in healthy volunteers compared with the more heterogeneous target population. Thus, mean parameter estimates could be comparable, but there could still be unexpectedly high incidences of adverse events or therapeutic failure in some patients due to too high or low drug levels, respectively. In addition, the variability in a parameter between occasions might be higher in patients because of disease progression factors.
- Healthy volunteers or selected patients are included in early clinical studies, in which the first pharmacokinetic data (and sometimes PK/PD) in man is evaluated. These results are then used as support for dose selection in later phases, which might result in less suitable dosing regimens, given that there is considerable pharmacokinetic difference between volunteers and patients.

To assess the influence of a disease, mean parameter estimates or concentrations/exposure and their corresponding variability in volunteers should be compared with estimates from the patient population. However, when comparing results from separate studies (phase I vs. phase III) there might be confounding factors such as demographic dissimilarities, that

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should be taken into account, e.g., age or gender differences. Phase II trials often include highly selected patients, which might not reflect the proper target population. One problem arises when the phase III trial in the target population has not been designed to estimate pharmacokinetic parameters, but supply, e.g., single trough concentrations, along with mixed inter- and intra-individual variability estimates. In these cases, comparisons are less reliable since the trough levels could be influenced by different dosing regimen, sampling, and assay error and may not represent the "true" concentrations [2]. If data from volunteers and patients are pooled, important patho-physiological factors can be included as covariates. Subgroups suffering from additional diseases (e.g., obesity) could be separately analyzed and compared with the total population, but the number of subgroup patients needs to be sufficiently large.

Impact of Comorbidity

The pharmacokinetics of the drug is evaluated in the target population fulfilling the criteria for which the indication is sought. The target population may be very wide and include subpopulations suffering from additional diseases affecting the pharmacokinetics of the drugs. These patients might not at all be represented in the trials or in too low numbers, not allowing their altered pharmacokinetic characteristics to be detected. It would be useful to know the kinetics of drugs in a very large number of patho-physiological situations; however, it is clear that this knowledge requires multiple, long, and expensive studies, which cannot all be performed. Examples of therapeutic areas for which the intended population is wide and difficult to fully incorporate in the usual clinical trials are pain medications, antihypertensive drugs, and antibiotics. If important disease-related effects on the pharmacokinetics are detected for a certain patient population, the information should be included in the labeling and, if necessary, appropriate restrictions such as contraindication, warnings, or dose adjustment should be included in the labeling.

Examples

Altered pharmacokinetic characteristics have been reported in the literature for various diseases or conditions, some of which are briefly summarized below.

Circulatory Disorders. This term includes, for example, congestive heart failure and malignant hypertension, generally characterized by diminished organ perfusion. Acute cardiovascular failure reduces the perfusion of liver and kidney and hence CL of highly extracted drugs

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might be affected. The enteral absorption may be reduced due to diminished perfusion and occasionally increased back pressure on the gut. The volume of distribution might be increased. The kinetics of distribution is affected, with diminished perfusion rates to certain organs. Reduced perfusion may cause metabolic acidosis that can alter the distribution of ionized drugs [3, 4].

Obesity. Obese individuals are subjected to different drug treatments for which the dosage recommendations have not been specifically evaluated with respect to obesity. Obesity is likely to affect drug distribution and elimination, whereas absorption is less likely to be modified. Alterations that may occur in obesity are increased distribution volume due to drug tissue distribution, alteration of the drug metabolic activity and cardiac performance. It has turned out to be rather difficult to predict the impact of obesity based on its lipophilic characteristics when it comes to markedly lipophilic drugs, whereas more hydrophilic drugs are more predictable, possibly due to their distribution mainly to lean tissues. For a more lipophilic drug, changes in distribution volume might appear and then adjusting the loading dose to bodyweight should be considered [5].

GI-Disorders. Diseases in the GI-tract may affect different factors important for drug absorption, and the effect on the overall pharmacokinetics is not always predictable. Inflammatory bowel diseases, such as Crohn's or ulcerative colitis, affect the absorption surface area and there are several reports on altered absorption in patients suffering from these conditions [6]. In celiac disease, associated with stunted small intestinal villi and alteration of gastric emptying and pH, the intestinal CYP3A4 content was decreased [7]. Changes in pH (e.g., achlorhydria or AIDS gastropathy) might delay and reduce the absorption of pH-dependent drugs such as ketoconazole [6]. Changes in GI-motility, by e.g., irritable bowel syndrome (small intestine), diabetes mellitus and nonulcer dyspepsia (stomach), and idiopathic constipation (colon), may affect the absorption of orally administered drugs by changing the rate of delivery, bioavailability, or mucosal absorption. For poorly absorbed drugs both the rate and extent of absorption are likely to be altered, whereas for well-absorbed drugs an effect is mainly seen on the rate of absorption. Predictions are, however, complicated by factors such as drug-related properties, the formulation, and food effects [8].

Surgery. Some drugs are intended for postoperative treatment and hence the dosing recommendations are evaluated in the same population. However, also drugs unrelated to the surgery are used postoperatively, such as cardiovascular drugs. Absorption, distribution, and elimination of drug might be altered due to diminished gastric emptying, altered protein binding and renal impairment [9].

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Cystic Fibrosis. In patients with CF the absorption rate varies but the extent of absorption is generally not altered. There is a difference in distribution volume due to reduced lean body mass. Patients with CF have been associated with increased metabolic CL of many drugs. Increased activity of both phase I and II reactions have been demonstrated, although not all CYP-isoforms were affected. The renal CL of many drugs is enhanced, although no mechanistic explanation has been found [10].

Organ Transplantation. Following transplantation, patients undergo marked changes in the physiological functions associated with the transplanted organ. Drug absorption, distribution, and elimination may undergo time-dependent transition from that associated with organ failure to that of the normal state. A thorough understanding of how the pharmacokinetics is influenced is essential for optimal drug therapy and for improvement of long-term survival [11]. For sirolimus, indicated for prophylaxis of organ rejection in patients receiving a renal transplant, oral clearance was reduced and half-life prolonged in the patient population. The distribution volume was lower in patients as was also the blood to plasma partition ratio (data on file).

Conclusion

Disease-related differences in pharmacokinetics may give rise to exposure differences between volunteers and patients and may be responsible for part of the inter- and intra-individual variability within the target population. The importance of any pharmacokinetic changes is related to the therapeutic index of the drug and thus the therapeutic consequences of altered pharmacokinetics should be considered. The probability of a change in any pharmacokinetic parameter might be considered, e.g., the bioavailability may or may not be sensitive to a difference in absorption characteristics. If relevant changes are found and deemed as therapeutically important, these should be considered when designing and evaluating studies from which pharmacokinetics in HVs are extrapolated to patients, e.g., renal- and hepatic-impairment and interaction studies.

It is not possible to cover all patients in the clinical trials that in the future possibly will use the drug. Therefore, the only studies that should be submitted before marketing are those that seem necessary with regard to properties, indications, contraindications, routes of elimination, scheme of administration of the drug, and those required to define the necessary dose changes that cannot be calculated from the pharmacokinetic parameters available from HV and in patients without functional disturbance of absorption, distribution, and elimination systems [1].

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RENAL INSUFFICIENCY

Introduction

Renal excretion of drugs involves filtration, secretion, and reabsorption. The unbound fraction of a drug is filtered in the glomerulus. Also small proteins are freely filtered, but when the molecular weight of the protein exceeds 20,000 g/mole filtration falls sharply and filtration of albumin (molecular weight 69,000 g/mole) is very limited [3]. The filtration can be calculated by fu*GFR, where fu is the fraction unbound in plasma and GFR is the glomerular filtration rate, which in a 70 kg, 20-year-old man is about 120 ml/min. Drugs may also be secreted by active transport systems. These are predominantly located in the proximal tubule. If renal clearance, $CL_{R,}$ exceeds the filtration (CL_R>fu·GFR), both secretion and reabsorption may be involved, but secretion is more pronounced. Reabsorption is higher than secretion if renal clearance is less than the filtration (CL_R< fu·GFR). For the majority of exogenous compounds, reabsorption occurs by a passive process. Reabsorption occurs all along the nephron, although the majority is reabsorbed in the proximal tubule. Many proteins, especially low molecular weight proteins, are substantially filtered in the glomerulus, but not excreted in urine. These are metabolized by enzymes located in the brush border of the proximal tubule lumen. Catabolism of proteins continues until constituent amino acids are formed.

As described above, renal function consists of several mechanisms. These may be differently affected by factors that influence renal function, e.g., age and renal disease. In adults, renal function steadily decreases with age, starting by the fourth decade [12]. Both glomerular number and size decrease with increased age [13]. Glomerular filtration rate and tubular function are generally considered to decrease at a parallel rate with age [12, 14].

Effects of Reduced Renal Function on Pharmacokinetic Parameters

The excretion of many drugs can be affected by the presence of renal disease, and for drugs principally eliminated via the renal route, drug excretion is diminished in patients with reduced renal function. Reduced renal excretion is not the only change in drug disposition in patients with renal insufficiency. There may be changes in absorption, protein- and tissue binding, and distribution and hepatic metabolism [15]. In addition, pharmacodynamics may also be altered in renal impairment [16].

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Absorption and Bioavailability

Altered drug absorption may be a result of prolonged gastric emptying time and increased gastric pH [15]. Increased bioavailability has been reported in patients with renal insufficiency secondary to decreased first-pass metabolism.

Distribution

Changes in drug distribution may arise from either fluid retention or changes in the extent of protein binding in tissue and plasma [15]. The plasma protein binding of most acidic drugs is decreased in uraemic patients [17]. These drugs are often highly bound to albumin and any modifications in the binding may have large effects on the fraction unbound. The decreased protein binding may be caused by hypoalbuminaemia, accumulation of endogenous competitive displacing substances or decreased affinity of human serum albumin caused by alteration in the conformation or structural arrangement of albumin-binding sites [17]. Conversely, the protein binding of basic drugs may be differently affected in renal failure (increased, decreased, or unchanged binding) [15, 18].

Metabolism

The results of studies on the effect of renal impairment on hepatic drug metabolism are conflicting. Metabolism has been shown to be increased, decreased or be unaffected by renal failure [18, 19]. Different drugs metabolized by the same cytochrome P450 isoenzyme have been reported to be differently affected by renal impairment. For different beta-blockers metabolized by CYP2D6, metabolism has either been reported to be decreased or unchanged, and for different calcium channel-blockers metabolized by CYP3A4, metabolism has been reported to be increased, decreased, or unchanged [18]. Sulphatation and glucuronidation are generally normal, whereas *N*-acetylation of isoniazid has been reported to be reduced in chronic renal failure [15, 20]. Metabolic ratios of metabolite and drug excreted in urine are often used for phenotyping of polymorphic drug metabolizing enzymes as well as for estimations of enzyme activity. As renal clearance of the drug or metabolites may affect such ratios, the ratios may be different in patients with renal impairment than in the overall population.

The pharmacokinetics of drugs metabolized or catabolized in the kidneys, but not excreted in urine, such as peptides and small proteins, is affected by renal impairment. The elimination of these will be decreased, resulting in accumulation in renal impairment.

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Accumulation of Metabolites

Metabolites that are renally excreted will accumulate in renal impairment. This could lead to increased efficacy or toxicity for pharmacologically active or toxic metabolites. Also metabolites that are considered relatively inactive in patients with normal renal function may reach active/toxic levels if the accumulation of the metabolites is extensive in renal impairment.

Estimation of Renal Function

Renal function is usually assessed through calculation of glomerular filtration rate (GFR). The reference method for estimating GFR is inulin clearance. Inulin is an inert polysaccharide cleared exclusively by glomerular filtration. The method includes constant intravenous infusion of inulin and timed collection of urine and is not practical for routine clinical purposes. A number of alternative methods have been developed for estimation of GFR. Many involve collection of urine and may give inaccurate results unless collection of urine is complete, including complete emptying of the bladder. Several methods to determine the plasma clearance of a suitable exogenous marker have been developed. These include radionuclides such as ^{51}Cr-EDTA and ^{99m}Tc-DTPA (diethylenetriaminepentaacetic acid) [21]. Although these methods are accurate, the requirement of radiolabeled tracers complicates the procedure (complicated handling, storage, and disposal of waste) and excludes certain patients, such as pregnant women. Alternative nonlabel filtration markers include the exogenous markers iothalamate and iohexol [22, 23] or endogenous markers such as Cystacin C [22] and, most importantly, creatinine [24].

GFR can be estimated by calculating creatinine clearance (CLcr) utilizing the serum creatinine concentration (Scr) and other patient characteristics such as bodyweight, age, gender, and height. All methods for estimating CLcr from Scr are simple, but are limited. Prediction of CLcr will not be accurate unless renal function and serum creatinine are at steady state and is not accurate in patients with unusually low or high muscle mass, in patients with marked obesity or ascites [24], or in patients with liver disease [25]. Moreover, creatinine is not exclusively filtered, but also subject to tubular secretion. Thus, GFR is overestimated by CLcr. This is especially evident in severe renal impairment. Creatinine clearance can also be determined from serum creatinine concentration and urinary excretion of creatinine. With this method, some of the drawbacks of using Scr can be avoided. The results are more accurate than estimation from Scr alone if complete collection of urine, including complete emptying of the bladder, can be obtained.

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Given the limitations of using CLcr as a measure of renal function, more accurate methods for measuring renal function, such as ^{51}Cr-EDTA, iothalamate or iohexol, should be considered in clinical studies evaluating the influence of renal function on the pharmacokinetics of new drugs.

Classification of Renal Impairment

Renal function is usually classified as normal renal function (CLcr≥80 mL/min), mild renal impairment (≥50-<80 mL/min), moderate renal impairment (≥30 mL/min-<50 mL/min), severe renal impairment (<30 mL/min), and end-stage renal disease (patients requiring dialysis) [26]. However, dose adjustments should be based on the actual results and do not need to follow the classification.

Evaluation of Pharmacokinetics in Renal Impairment

When and How to Perform Pharmacokinetic Studies in Patients with Renal Impairment

The FDA guidance for industry "Pharmacokinetics in patients with impaired renal function—Study design, data analysis, and impact on dosing and labelling" [26] gives detailed information on the FDA requirements for when and how pharmacokinetic studies should be performed in patients with renal impairment. A corresponding European guideline is presently being written, but has not yet come into operation (2003) [27]. Although not yet formally specified in an approved guideline, the requirements in Europe for pharmacokinetic characterisation in patients with renal impairment are essentially similar to those of the FDA.

A pharmacokinetic study in patients with impaired renal function is recommended when renal impairment is likely to significantly alter the pharmacokinetics of a drug and/or its active/toxic metabolites, and a dosage adjustment may be needed for safe and effective use in such patients.

As described above, severe renal impairment has been associated with changes in absorption, hepatic metabolism, protein binding, and distribution also for drugs that not are excreted renally. Hence, pharmacokinetic characterization in patients with severe renal impairment should be considered also for drugs eliminated mainly by metabolism, in particular when the drug or its active metabolites exhibit a narrow therapeutic index.

Study Design

The primary goal of a study in patients with impaired renal function is to determine if the pharmacokinetics is altered to such an extent that the

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dosage should be adjusted from that established in the phase III trials, where efficacy and safety has been shown. Thus, the study should focus on comparing patients with renal impairment with patients with renal function that is typical of the clinical trial patient population—not necessarily with healthy young volunteers.

To ensure adequate representation of patients with various degrees of renal impairment, approximately equal numbers of patients from each of the renal impairment groups (normal renal function, mild, moderate, severe renal impairment, end stage renal disease) should be recruited. The renal function groups should be comparable with respect to age, gender, and weight and other factors with significant potential to affect the pharmacokinetics of the drug (e.g., diet, smoking, alcohol intake, concomitant medications, ethnicity). The number of patients enrolled should be sufficient to detect clinically relevant pharmacokinetic differences.

If there is good reason to believe that renal impairment does not affect the pharmacokinetics to a degree sufficient to warrant dose adjustment, it may be sufficient to study only patients at the extremes of renal function (i.e., patients with normal and severely impaired renal function). If the results confirm that renal impairment does not relevantly alter the pharmacokinetics, no further study is warranted. If the results do not strongly support such a conclusion, the intermediate renal function groups (mild and moderate renal impairment) should also be studied.

A population pharmacokinetic evaluation of patients participating in phase II/phase III clinical trials may be used to assess the impact of renal function on the pharmacokinetics of a drug. In principle, such a population pharmacokinetic study design and analysis can be an acceptable alternative to a specific renal impairment study if:

- it includes a sufficient number of patients and a sufficient representation and range of renal function so that the study could detect relevant pharmacokinetic differences
- unbound concentrations have been measured, when appropriate
- both parent drug and potentially active/toxic metabolites are measured, when appropriate.

Patients with severe renal impairment are often excluded or poorly represented in population pharmacokinetic studies. When that is the case for a drug likely to be administered to such patients, a separate and complementary study could be conducted to assess the pharmacokinetics in patients with severe renal impairment (e.g., a study evaluating the pharmacokinetics in subjects with severely impaired renal function compared with subjects with renal function typical for the phase III population). The data from both sources should be used in the overall assessment of the effect of renal impairment. Even if the above requirements

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regarding unbound concentrations and analysis of metabolites cannot be fulfilled for samples collected in phase II/III studies and the population analysis cannot replace a conventional study, population analysis of the impact of renal function on the pharmacokinetics in the target population is recommended, as this provides valuable information regarding variability in the target population. These data could be evaluated in conjunction with the data from a conventional renal impairment study.

Dialysis

Dialysis may significantly alter the pharmacokinetics of drugs. When a significant fraction of the drug or active metabolite(s) is removed by dialysis, a change in the dosing regimen may be required, such as a supplementary dose following the dialysis procedure. It should be remembered that also drugs that are not excreted by the renal route to a large extent may be removed by dialysis.

For drugs that are likely to be administered to end-stage renal disease (ESRD) patients undergoing dialysis and where the drug or active metabolites are likely to be extracted during dialysis to such an extent that supplementary dosing after dialysis may be required, evaluation of the pharmacokinetics under both dialysis and nondialysis conditions should be considered in order to determine the contribution of dialysis to the elimination of the drug and potentially active metabolites.

Presentation and Evaluation of Results

In the presentation of results, a graphical description of the relationship between individual pharmacokinetic parameters and renal function should be included. This is important for assessment of the variability in normal and reduced renal function and facilitates the identification of cut-off for dose adjustment. The FDA guideline emphasises the construction of mathematical models to evaluate the relationship between renal function and pharmacokinetic parameters. Although renal clearance of many drugs decreases with reduced renal function, the relationship between renal function and pharmacokinetic parameters is not necessarily linear. Descriptive statistics of the pharmacokinetic parameters according to renal function (normal, mild, moderate, and severe renal impairment) can also be presented.

Study results including the model for the relationships between renal function and relevant pharmacokinetic parameters should be used to construct specific dosing recommendations. Typically, the dose is adjusted to produce a comparable range of unbound plasma concentrations of drug or active metabolites in both normal patients and patients with impaired renal function. As discussed above, it is important to consider the variability in pharmacokinetics in renal impairment, the therapeutic index and consequences of reduced and increased exposure,

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respectively, in this assessment. A recently proposed approach is to estimate appropriate cutoffs and doses, given the information on pharmacokinetic parameters and distribution of renal function in the population [28]. Regardless of whether specific dose reductions are recommended or not, simulations of the steadystate exposure and predicted variability at the proposed dose(s) is a valuable tool for confirming the suitability of the chosen posology.

Current Experience and Ways Forward

Generally, renal impairment studies are fairly well performed, but there is room for improvement. Deficiencies still seen in these studies include poorly presented results and dose adjustments based on mean data without taking variability into account. There is room for improvement in these areas. Also, the use of unbound exposure, when applicable, and population pharmacokinetics is likely to increase. In the future, increased evaluation is expected of the influence of renal impairment on the pharmacokinetics of renally excreted metabolites, both active and inactive, and hopefully more studies will be performed evaluating the effect of renal impairment on active transporter systems.

FDA has published a survey of renal impairment studies performed during 1996 and 1997 [29]. The survey indicated that in the past, no consistent pharmacokinetic property drove the decision to conduct renal impairment studies, there was no consistency in study design, number of groups of patients with reduced renal function, and the number of patients/group. In most protocols 24 h CLcr was used to assess renal function, in 75% of the studies the doses used were in the therapeutic range, a point estimate with ANOVA was generally used to analyze data and there was no consistent method for presenting data from renal impairment studies in the product labeling. In part, based on this survey FDA developed the guidance for studies of pharmacokinetics in renal impairment to promote welldesigned studies with adequate presentation of results resulting in consistency in product labeling.

LIVER DISEASE

Introduction

The pharmacokinetics of drugs may be altered in liver disease. This primarily applies to drugs that are eliminated by the liver to a substantial extent although drugs that are eliminated by other organs may be affected through effects secondary to the hepatic impairment. Possible causes of the changed pharmacokinetics are several, including reduced enzyme activity,

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altered hepatic blood-flow, shunting of blood past the liver, decreased protein binding and secondary renal impairment. To what extent the drug is affected by the hepatic impairment is dependent on the pharmacokinetic properties.

Liver disease is a heterogeneous group of diseases with different morphological changes and symptoms. Presently there is no optimal marker for assessing hepatic function. Therefore it is difficult to predict the pharmacokinetics of a certain drug in individual patients as well as making extrapolations to nonstudied types of liver diseases. Recently the U.S. FDA has issued a guidance for pharmacokinetics in patients with impaired hepatic function [30] and an EU guideline is under preparation [31].

Liver Diseases—A Variety of Conditions

There are numerous reasons for impairment of the hepatic function. In the western world, chronic alcohol abuse is one of the main causes of liver disease and can cause steatosis, alcohol hepatitis, and cirrhosis.

Steatosis is a condition caused by disturbances in the lipid metabolism and produce an accumulation of triglycerides within the hepatocytes. The condition may appear quickly and is reversible if the cause of the accumulation is removed. The condition is mainly caused by alcohol but may also be caused by malnutrition, hepatotoxic substances, diabetes, and obesity.

Hepatitis is mainly caused by viruses, hepatotoxic substances, and autoimmune diseases. The condition is characterized by cell necrosis and inflammation in the liver. All forms give the same alterations of the liver, including simultaneous necrosis and degeneration of hepatocytes, infiltration of mononuclear cells, degeneration of Kupffer cells, and varying degree of cholestasis.

Cirrhosis is not a disease in itself but a stage in the course of inflammatory liver diseases. Cirrhosis can be caused by liver damage resulting from alcoholism, hepatitis B and hepatitis C, drugs, metabolic disorders, prolonged cholestasis, etc. Cirrhosis is characterized by increased presence of fibrous tissue, destruction of the lobular architecture and sinusoidal network, and nodular degeneration. The hepatic synthesis of proteins such as albumin, prothrombin, and enzymes is decreased. Cirrhosis often gives rise to portal hypertension. In portal hypertension, the blood flow coming from the intestine through the liver via vena porta is reduced while the arterial blood flow is increased relative to the portal flow. Many cirrhotic patients have portacaval shunts, where a substantial fraction of the portal blood bypasses parenchymal tissue in the liver or enters directly into the superior vena cava via esophageal varices. A characteristic late sign of liver disease is ascites, an accumulation of extracellular fluids in the lower

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abdominal area. Ascites is believed to be caused by a combination of portal hypertension and decreased colloid osmotic pressure in the blood. The degree of portal hypertension, shunting, ascites, and residual metabolic capacity varies between cirrhotic patients.

Usually pharmacokinetic studies are performed in cirrhotic patients. As there is no optimal marker(s) for assessing hepatic function, extrapolation from study results to individual cirrhotic patients as well as to patients not having cirrhosis is problematic.

Estimates of Liver Function

Child-Turcotte classification is an empirical but commonly accepted way to estimate the grade of cirrhosis even though it is not known to what extent it may be used to estimate hepatic function. In 1973, Pugh used Child's classification system but added the prothrombin time when he wanted to classify patients in a study with regard to the risk related to surgery of the oesophagus [32]. Since then, the degree of liver dysfunction is determined mainly by ranking the patient according to the Child-Pugh classification. Using this classification, the patients are grouped into mild, moderate, or severe impairment based on both two clinical symptoms of liver disease (encephalopathy and ascites) and three clinical chemistry parameters (S-albumin, S-bilirubin, and prothrombin time) (Table 1).

Based on the Child-Pugh scores, the patients are divided into groups called A, B, C, or "Mild", "Moderate", or "Severe" corresponding to 5–6, 7–9, and 10–15 scores, respectively. As a result, patients with a normal hepatic function are given a total score of five points and would consequently be classified as having mild liver impairment.

In the majority of pharmacokinetic studies, the Child-Pugh classification is used to assess the degree of liver function impairment. In patients evaluated for classification purpose, it is important that impaired hepatic function and not some other underlying disease is the cause of alterations in the Child-Pugh components. For example, in patients with metastatic cancer, hypoalbuminemia, encephalopathy, and ascites may be related to cancer cachexia or cancer metastatic to the brain or peritoneal surfaces rather than impaired hepatic function. The Child-Pugh classification is not an optimal estimate of liver function with respect to drug elimination capacity and research is presently going on trying to find alternative markers. Several markers including antipyrine, trimethadione, caffeine, lidocaine, midazolam, etc., have been tried. Trimethadione, for example, has been used in the clinic for assessing the function of the liver before and after liver-transplantation [33]. Such a marker may be a useful tool for dose-adjustments and could be used alone or in parallel with the Child-Pugh classification. Until better markers have been found, the

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TABLE 1 Ranking of Liver Dysfunction According to the Child-Pugh Score

Assessment	Degree of Abnormality	Score
Encephalopathy	None	1
	Moderate	2
	Severe	3
Ascites	Absent	1
	Slight	2
	Moderate	3
S-Bilirubin (mg/dL)	<2	1
	2.1–3	2
	>3	3
S-Albumin (g/dL)	>3.5	1
	2.8–3.5	2
	<2.8	3
Prothrombin time (sec.> control)	0–3.9	1
	4–6	2
	>6	3

Child-Pugh classification system can be used to categorise the degree of hepatic impairment of patients included in a pharmacokinetic study and can, together with its individual components, be used when evaluating the pharmacokinetic results.

To make the dose-adjustments more precise, attempts could be made to find a clinically available marker that is better correlated with the exposure (AUC and Cmax) than the Child-Pugh classification. Below is an example where we tried to correlate the observed exposure not only to Child-Pugh score but also to the separate clinical chemical parameter included in this classification system (Figs. 1 and 2). S-albumin was the parameter that was best correlated with exposure (AUC) of drug. It is, however, recognized that S-albumin is affected also by other conditions and is not useful as the only estimate of liver function in patients.

Extrapolations from Cirrhosis to Other Liver Diseases

Although liver disease is a heterogeneous group of diseases, the pharmacokinetics of a new drug is often limited to studies in cirrhotic patients. As this is the most common liver disease, this is of benefit for the

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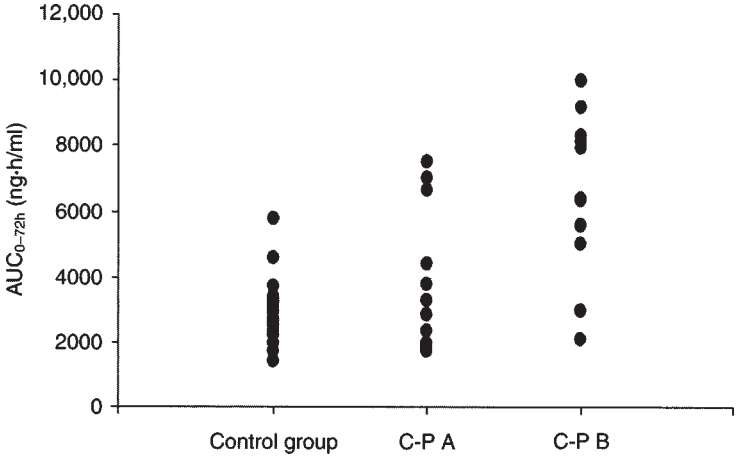

FIGURE 1 AUC of an antiinflammatory drug in patients with different degrees of liver function.

majority of patients. However, extrapolating to patients with other liver diseases may be difficult. Different kinds of liver diseases may affect the pharmacokinetics of a drug differently. For example cholestatic and noncholestatic cirrhosis appear to affect the enzyme expression and/or availability of specific enzymes in different ways [34, 35]. The amount of

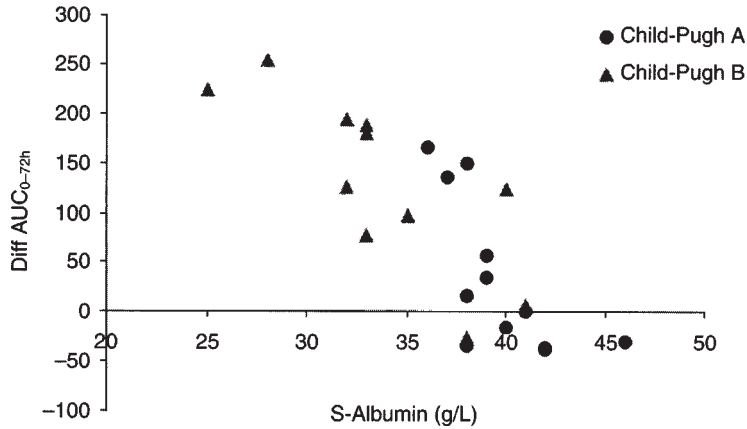

FIGURE 2 Difference in AUC (%) in patients with liver impairment as compared with matched healthy volunteers vs. S-Albumin.

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CYP1A2 appears to be decreased in both hepatocellular and cholestatic cirrhosis while the levels of CYP3A were only observed to decrease in patients with hepatocellular cirrhosis. The levels of CYP2E1 were reduced in patients with cholestatic cirrhosis while the decrease was seen at mRNA- but not protein-level in livers of patients with hepatocellular cirrhosis [34]. In contrast, markedly (5–10-fold) increases in CYP2E1 levels have been observed in alcoholics [36, 37]. Due to the discrepancies in effects of the different diseases, it is important to give information in the labeling regarding which population has been studied. If new markers of liver function are found, a safer and more precise extrapolation from cirrhosis to other diseases could be possible.

Effects of Impaired Liver Function on Pharmacokinetic Parameters

Presently, hepatic extraction and clearance are usually assumed to proceed according to the "well-stirred model" [3]. In this model, the liver is assumed to work as a well-stirred compartment where the drug and enzymes are evenly distributed. When predicting how the pharmacokinetics is affected by altered physiological conditions, it should be remembered that a simplified model of the liver is used.

Altered Hepatic Blood Flow

The hepatic blood-flow may be decreased in cirrhosis but predictions of the effect on pharmacokinetic parameters are ambiguous. Reduced blood flow and shunting can both increase the bioavailability of drugs subject to hepatic first pass metabolism and also reduce the systemic hepatic clearance of drugs depending on their extraction ratio [3].

Alterations in Enzyme Activity

Drug metabolism catalyzed by cytochrome P450 enzymes is generally decreased in cirrhosis whereas it may, at present, be less predictable and have been less investigated in other liver diseases. The reduced metabolism in cirrhosis is probably due both to reduced viable cell-mass and as well as reduced enzyme synthesis in the hepatocytes. Decreases have been observed in mRNA-level and protein-level as well as in enzyme activity [34, 35]. The sensitivity to liver disease appears to vary between enzymes [38]. Drug metabolism catalyzed by CYP2C19 appears to be markedly decreased in patients with cirrhosis while the CYP2D6 activity seems less affected [39]. In general, the UDP-glucuronosyl transferase enzymes appear less affected than the cytochrome P450 enzymes although the sensitivity to liver disease differs between isoforms [40, 41]. The

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pharmacokinetic consequences of a decrease in enzyme activity depend on the pharmacokinetic characteristics, e.g., extraction ratio and contribution of liver metabolism to elimination of the drug [3].

Altered Plasma Protein Binding

Due to a depressed synthesis of albumin in the liver, the protein binding of drugs may be decreased in cirrhosis. This may have consequences both for the elimination and the distribution of drugs. The magnitude of the effect on elimination is again dependent on the pharmacokinetic characteristics of the drug [3].

Secondary Renal Failure

During the clinical course of cirrhosis, secondary changes in the kidneys may give rise to renal insufficiency. The renal perfusion can be decreased and the reabsorption of sodium in the proximal tubule is increased in decompensated cirrhosis. In addition, in patients with decompensated cirrhosis, serum creatinine and creatinine clearance estimated from serum creatinine are not sensitive markers for renal function and often overestimate actual GFR [42]. This may be caused by a reduced hepatic production of creatine, the precursor of creatinine, or a reduced conversion of creatine to creatinine due to decreased muscle mass [43].

Evaluation of Pharmacokinetics in Hepatic Impairment

The effects of liver disease on the pharmacokinetics of a drug should be investigated if hepatic metabolism or excretion contributes to a substantial part of the total elimination and/or if an active metabolite is formed or eliminated by the liver. In addition, studies may be considered if the drug is extensively protein-bound or if it has a narrow therapeutic range. The main objective of a hepatic impairment study is to identify patients at risk and, when appropriate, to develop dosing recommendations in the patients with hepatic disease.

The effect of liver disease on the pharmacokinetics of a drug is usually investigated in cirrhotic volunteers. The diagnosis should, if possible, be established by biopsies. The group of cirrhotic volunteers should generally cover the whole range of metabolic impairment. A control group should be included representing the target population with respect to demographic factors. The hepatic function groups should be comparable with respect to age, gender, weight, and other factors with significant potential to affect the pharmacokinetics. The use of historical controls instead of including controls with normal liver function is not recommended as, due to

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interstudy variability, this may mask a difference in pharmacokinetics of the drug. The number of volunteers or patients included should be sufficient to detect clinically relevant pharmacokinetic differences.

Patients classified by the Child-Pugh system as having mild impairment could have a normal hepatic function and for the majority of drugs, clinically significant differences are more likely to be observed in patients with moderate and severe impairment. Thus, a reduced design including only patients with moderate impairment and controls may be used to screen for significant effects. If a significant effect is detected in the moderate group, the pharmacokinetics in patients with mild impairment needs to be evaluated to propose dose recommendations for this group.

An alternative way of assessing the effect of liver disease on the pharmacokinetics of a drug is to use population pharmacokinetic data obtained in phase II and III studies as has been described for renal impairment. However, this approach may prove more difficult here due to e.g., lower prevalence of hepatic impairment in the general population. In these studies, patients with hepatic impairment should be identified and classified using the same criteria as has been discussed for the conventional studies. Population analysis for this purpose should be prespecified.

For prodrugs (i.e., drugs with activity predominantly due to hepatically generated metabolite), it is possible that the dose may need to be increased, or the dosing interval shortened, in hepatically impaired patients.

Ways Forward/Room for Improvement

As discussed above, the presently used Child-Pugh classification is not optimal for assessment of drug-elimination capacity and it would be useful to find markers better reflecting the different hepatic elimination mechanisms. Markers like serum albumin, prothrombin time, and bilirubin may be more related to drug elimination capacity than other components of the Child-Pugh scale. An ideal marker should be proven relevant and should preferably not be affected by other conditions. The reason for lack of effect on the pharmacokinetics may be due to inclusion of subjects in whom, although classified as having hepatic impairment, the elimination capacity for the drug is not altered. One way to ensure that the included subjects have impaired metabolic capacity may be to administer a probe drug (e.g., CYP3A4 probe if the compound being investigated is a CYP3A4-substrate) to confirm that an effect would be detectable in the studied subjects. In the future, more specific markers may ensure reliable identification of patients at risk and support proper dosing recommendations to patients with different degrees of hepatic impairment.

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INTERPRETATION OF DATA

Marketing applications for new medicinal products often include studies in adequate subgroups of patients. The aim is to develop dosing recommendations that will decrease the overall variability in the population and ensure that the patient will obtain treatment that is effective and safe. Factors that should be taken into account are the intended use of the drug, the pharmacokinetic characteristics of the drug and the PK/PD relationship regarding efficacy and safety. Based on available information regarding the latter, target criteria should be specified a priori for what change in the pharmacokinetics would justify a posology adjustment. The target criteria should be based on the major concern (side effect or lack of efficacy) for the specific product. It is not uncommon that not only the mean exposure is increased in specific subgroups but also the inter-individual variability. In a group of patients with moderate hepatic impairment, some patients may show no increase in exposure at all, while others show a significant increase. Again, what is our main concern—concentration-dependent adverse events or subtherapeutic level?

When investigating the pharmacokinetics in patients with decreased organ function the most common approach is to study patients with various degrees of impairment. To ensure that a sufficient number of patients are included, patients are often stratified according to predefined criteria into mild, moderate, and severe impairment. Unfortunately, it is not uncommon that data are presented as mean values +/- S.D. within these subgroups. However, when assessing the results from such data, there is no reason to use these predefined criteria. As has been pointed out above, the entire information available should be utilized.

In a group of patients with a reduced elimination of the drug compared with other patients it is often impossible to provide a dosing recommendation resulting in identical concentration-time profiles. Regardless of whether the dose is reduced and/or the dosing interval is increased, one needs to focus on either similar AUC or similar Cmax. Similar AUC often results in lower Cmax, while similar Cmax results in higher exposure in terms of AUC and Cmin. Again, knowledge of the PK-PD relationships is needed to make these decisions.

Finally, when studying an effect of a disease state on the pharmacokinetics, the reference group is often healthy volunteers. It should be remembered that the phase III population might be more similar to the test population than the reference group. A product developed for Alzheimer's disease showed increased exposure in patients with mild to moderate renal impairment compared with healthy volunteers. The magnitude of this difference was such that a dose reduction may be considered. However, a careful look at the phase III population, where an

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effective and safe dose had been established, showed that the majority of patients had a creatinine clearance corresponding to a mild to moderate renal impairment. In fact, the dose should possibly be increased in patients with a relatively high filtration clearance to avoid subtherapeutic levels.

LABELING

With the aim to provide clear guidance to the prescriber, sponsors and regulatory agencies may run a risk of simplifying the situation too much. When deciding on a wording there may be a tendency to contraindicate the use in a subgroup of patients when no information is available, but to generalize too wide when limited data are provided. In the former situation, no extrapolation from the general PK characteristics is allowed, while this is acceptable in the latter case.

Hepatic impairment is an illustrative example of this. A drug that is eliminated through metabolism may be contraindicated in patients with moderate to severe impairment if no data are available (regardless of therapeutic margin). If the sponsor provides a study with a low number of cirrhotic patients Child-Pugh A and B, the labeling could well read "Patients with mild to moderate hepatic impairment should be given half the recommended maintenance dose." This occurs despite the fact that only cirrhotic patients were studied, that only a few were moderate according to Child-Pugh, that there was a considerable variability in the exposure in this group of patients, and that we know that the correlation between Child-Pugh classification and metabolic capacity is poor. The way forward is to accept that we sometimes cannot give clear guidance. When this is not possible we should provide the prescriber with the information available. This could include general pharmacokinetic characteristics of relevance for the subgroup together with available specific information including the type of patients in which the information was obtained (e.g., cirrhotic patients). The prescriber can then decide what to do without being faced with a contraindication based more on "lack of data" than a real clinical concern.

For more detailed guidance recommendations, readers are encouraged to refer to the Guidelines on renal and hepatic impairment from FDA [26, 30] and EU (CPMP) [27, 31].

CONCLUSIONS

It is unreasonable to require that efficacy and safety is established in phase III studies including all subpopulations that could be treated with a new medicinal product once on the market. To limit the size of these large

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comparative studies, we must accept that measures (i.e., inclusion/exclusion criteria) are taken to reduce the interindividual variability. Hence, we must use other tools, such as pharmacokinetic, pharmacodynamic, or animal studies to predict safety and efficacy in these patients. When this is not possible or a risk is identified, the prescriber must be informed in a proper way.

Globally, there are a number of regulatory guidelines discussing studies in subgroups of patients. Accordingly, the marketing applications submitted to regulatory agencies today often include studies in relevant subgroups. This may result in specific dosing recommendations. Without that information, regulatory agencies may have elected to contraindicate that subgroup.

Given the discussions in this chapter, the obvious question is if we are simplifying the matter too much today? Possibly sponsors are not taking full advantage of their scientific expertise when designing and interpreting the results from these studies? And perhaps regulatory agencies are too willing to contraindicate subgroups when information is not available and extrapolate too widely when some, but perhaps insufficient information is present?

If this is the case, it is in the interest of the patient to stimulate sponsors to perform better scientific studies and to provide prescribers with more precise information about available knowledge. This would put them in a better position when deciding if and how to treat an individual patient.

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Clinical Pharmacology Issues Related to Specific Drug Classes During Drug Development

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INTRODUCTION

Clinical pharmacology plays a role throughout the development process of drugs in all therapeutic classes. Three conferences convened during the 1990s addressed the utility of clinical pharmacology information in the drug development process. The first conference, "The Integration of Pharmacokinetic, Pharmacodynamic, and Toxicokinetic Principles in Rational Drug Development," occurred in 1991 in Arlington Virginia. The other meetings were held in 1998: "AAPS, ACCP, ASCPT, FDA Symposium on Clinical Pharmacology: Optimizing the Science of Drug Development" in Arlington, Virginia, and "5th EUFEPS Conference on Optimizing Drug Development: Fast Tracking into Human," in Wiesbaden, Germany. The

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conference report for the 1991 meeting indicates that the coordinated application of pharmacokinetics and pharmacodynamics provides a rationale approach to efficient and informative drug development [1]. The report for the two 1998 conferences states that there are a number of opportunities for the use of clinical pharmacology principles at every step of the drug development process. Appropriate use of clinical pharmacology information allows one to identify and develop the best drugs with low risk potential and also to identify failures faster [2]. Earlier chapters in this book (Chapters 1, 2, and 4) elaborate on the utility of clinical pharmacology in drug development.

The basic clinical pharmacology issues are similar across drug classes and therapeutic indications. The ultimate goals are to understand the relationship between exposure and response and to determine factors that may alter exposure and response. Chapters 11, 12, 13, 15, and 16 describe in detail how one achieves these goals. As a summary, the following four steps describe the process.

Step 1: Determine desired efficacy endpoint
Step 2: Determine the relationship between exposure and response
Step 3: Determine dosing regimens that achieve the target concentration range
Step 4: Determine factors that alter drug concentrations

The steps outlined above allow one to identify a dosing regimen to evaluate for safety and efficacy and determine whether there are subpopulations that need different doses. In addition, there are several other situations where it is useful to understand the relationship between exposure and response for a particular drug. These situations include: the development of new formulations that are not bioequivalent to the approved formulation; changing a dosing regimen to allow for less frequent dosing; determining appropriate dose adjustments due to drug interactions; and extrapolating drug efficacy and safety data from adults to pediatric patients.

The following sections describe specific clinical pharmacology and exposure-response considerations for a number of drug classes. In all cases the goals are the same—to understand the relationship between exposure and response and determine factors that may alter exposure and response. However, depending on disease and drug characteristics, the utility of the information and the specific situations in which the information is used may differ. Also, the initial source of information that contributes to the exposure-response evaluation differs by drug class. In some cases there are good animal and in vitro models, in other cases only human data are useful. For some indications, studies in healthy volunteers provide information about drug activity, while other indications require patients for all efficacy studies.

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This chapter includes two groups of narratives on clinical pharmacology issues related to specific drug classes and indications. The first group of narratives includes detailed descriptions of clinical pharmacology issues, including exposure-response examples, for the following drug classes and indications:

Human immunodeficiency virus (HIV) infection
Antibiotics
Stroke and cerebrovascular diseases
Migraine
Gastric acid related disorders

The second group of narratives includes short descriptions of special issues for several drug classes and indications:

Neuromuscular blocking agents
Cancer chemoprevention
Antihypertensive agents
Inhalation drugs for pulmonary indications
Acute pain—the dental pain model
Immunosuppressive agents
Opioid analgesic agents
Lipid lowering agents

This chapter does not provide a prescriptive description of how to use clinical pharmacology to develop drugs in specific drug classes. Also, there are numerous indications and drug classes not covered in this chapter. However, the selected narratives provide a broad range of examples of important clinical pharmacology issues for specific drug classes. The issues covered in this chapter can be extrapolated to other drug classes and indications, as discussed in the chapter conclusion.

DETAILED DESCRIPTIONS OF CLINICAL PHARMACOLOGY ISSUES FOR SPECIFIC DRUG CLASSES

Human Immunodeficiency Virus (HIV) Infection

The clinical course of HIV infection includes primary infection (acute antiretroviral syndrome), asymptomatic infection, early symptomatic infection, and advanced immunodeficiency with opportunistic complications [3]. HIV RNA and CD4+ cell count are two laboratory tests that indicate the clinical status of a patient. The measurement of HIV RNA in plasma, also called viral load or viremia, indicates the amount of virus circulating in the patient's plasma. The number of CD4+ lymphocytes

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(CD4+ cell count) reflects patient immune status. As the viral load increases and CD4+ cell count decreases, the risk of opportunistic infections, malignancies, wasting, neurologic complications, and death increases [4]. In July 1997, the Antiviral Drug Products Advisory Committee concurred that favorable treatment-induced changes in HIV RNA levels are highly predictive of meaningful clinical benefit and that HIV RNA measurements may serve as endpoints in trials supporting accelerated and traditional approvals. In addition, changes in CD4+ cell counts should be consistent with observed HIV RNA changes [5].

The complexity of treating HIV leads to many situations where exposure-response information is useful. Most patients take three or more antiretroviral drugs per day, in addition to drugs that treat or prevent opportunistic infections and treat complications of the antiretroviral agents, so there is the potential for many drug interactions. Many of the drugs are administered two or three times per day; some drugs have stringent food restrictions. Exposure-response information helps determine appropriate dose and regimen adjustments when drugs interact with each other and when food alters exposure. Due to the large pill burden, drug companies want to use exposure-response information to support changes in formulations and dosing regimens. For example, a drug company may want to change a dosing regimen from three times per day to two times per day. When making such a change for a drug with dose-proportional pharmacokinetics, the twice daily regimen will provide similar total exposure to the drug (area under the concentration vs. time curve [AUC] over 24 hours) as the three times daily regimen, but trough concentration (concentration at the end of a dosing interval) will be lower and C_{max} (maximum concentration) will be higher. If adequate exposure-response data are available, the drug company may use it to provide evidence that the lower trough concentration will not compromise efficacy and the higher C_{max} will not cause unacceptable toxicity.

Various investigators have evaluated exposure-response relationships for different classes of antiretroviral agents. A majority of the evaluations focus on the first three approved classes of drugs—nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs).

NRTIs inhibit viral replication by interfering with the DNA polymerase function of viral reverse transcriptase. After uptake by host cells, nucleoside analogues are converted to their active triphosphate forms by cellular kinases [6]. The population pharmacokinetics and pharmacodynamics of abacavir, an NRTI, were investigated in 41 HIV-1 infected antiretroviral naïve adults [7]. Patients received blinded monotherapy with abacavir at 100, 300, or 600 mg twice daily for up to 12 weeks. The efficacy measures used in the analysis were time-averaged changes in HIV-1 RNA and CD4+

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cell count. The investigators used standard Emax and sigmoid Emax models to evaluate exposure-response relationships for patients who completed 12 weeks of mono therapy (n=21).

The exposure-response evaluations indicated changes from baseline values in both time-averaged HIV-1 RNA level and CD4+ cell count were associated with abacavir $AUC_{0-\infty}$. There was also a relationship between the efficacy parameters and abacavir Cmax, but the relationship was not as strong as that with AUG. The EC_{50} value for the time-averaged change in HIV-RNA level was greater than that for the CD4+ cell count, indicating early saturation of the CD4+ cell count change. There was a modest increase in HIV-1 RNA suppression, but no increase in the CD4+cell count, observed at 600 mg twice daily relative to 300 mg twice daily as monotherapy. The results from this evaluation supported the further evaluation of 300 mg twice daily for the treatment of HIV infection.

The protease inhibitors (PIs) are associated with dramatic improvements in immune function and decreases in viral load. Inhibition of the protease enzyme results in the release of noninfectious and immature viral particles [8]. The relationship between plasma indinavir concentrations and changes in HIV RNA was evaluated in 23 protease inhibitor naïve patients [9]. Patients received indinavir 800 mg three times daily, in combination with NRTIs. There was significant interpatient variability in indinavir AUC_8, values ranging from 5.4 to 52.3 μM*hr. As indicated in Table 1, median AUC_8 and trough concentrations (C_8) were higher in patients with undetectable HIV RNA (<500 copies/mL) compared to those patients with detectable HIV RNA. However, there is a great deal of overlap in values between the two groups.

These results indicate that variability in plasma drug concentrations contributes to the variability in response. Thus, drug interactions or dosing regimen changes that lead to lower indinavir concentrations may have a negative impact on efficacy. In spite of this observation, the investigators did not determine a threshold indinavir concentration necessary for efficacy. Also, there is much variability in drug response that plasma drug concentrations do not explain.

TABLE 1 Median (range) Indinavir Exposure Measure in Patients with Detectable and Undetectable Plasma HIV RNA

Parameter	Detectable Plasma HIV RNA ($n = 9$)	Undetectable Plasma HIV RNA ($n = 14$)
AUC_8 (μM*hr)	22.4 (5.4–35.6)	30.7 (7.9–52.3)
C_8 (μM)	0.04 (0.02–0.16)	0.24 (0.02–3.4)

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For simplicity, the examples above focus on information about the relationship between exposure and efficacy. However, safety is an important response measure. Safety concerns with NRTIs include anemia, pancreatitis, peripheral neuropathy, and lactic acidosis. Certain NNRTIs are associated with rash, liver toxicity, and CNS side effects. PIs contribute to hypertriglyceridemia, hyperlipidemia, fat redistribution, and diabetes. Many antiretroviral agents cause gastrointestinal adverse events. Adding exposure-response evaluations for adverse events to the assessment for efficacy adds a layer of complexity.

Although numerous investigators have evaluated relationships between exposure and response for antiretroviral agents, there is no definitive conclusion regarding the specific exposure measures that correlate with efficacy or safety for each drug class. Based on the scientific principle that maintaining plasma concentrations above a threshold necessary to inhibit viral replication (e.g., in vitro IC_{50} or IC_{90}—concentration of a drug that inhibits viral replication by 50 or 90%, respectively) throughout an entire dosing interval is essential, many investigators believe that the minimum plasma concentration *(C_{min})* is the most important exposure measure for predicting success with PIs and NNRTIs. This concept is based on knowledge about the viral kinetics of HIV, which predict that suboptimal concentrations of antiretroviral drugs result in the production of large numbers of virions under conditions of high selective pressure. This situation may put patients at risk of eventual virologic failure due to the emergence of mutant HIV strains. Although the concept that C_{min} is the most important pharmacokinetic parameter is highly plausible, clinical data have not confirmed it. For NRTIs, it is important to consider moieties other than parent drug, because the intracellular triphosphate form of the drug is active.

One limitation that complicates the evaluation of exposure-response for antiviral agents is the fact that antiviral efficacy may change over time. This change may occur because the virus develops resistance to the various drugs; thus, the effective concentration may increase over time. This factor makes it difficult to draw definitive conclusions from short-term studies. Concerns about changes in viral susceptibility also lead one to consider whether relationships determined for patients who are antiretroviral naïve will be the same as relationships developed for patients who have received a lot of prior therapy and may have higher baseline drug resistance.

Combination antiretroviral therapy that includes a PI is associated with dramatic improvements in immune function and decreases in viral load. However, there are a number of factors that limit the success of therapy with PIs. Some of the factors include high first-pass metabolism by CYP3A, efflux by P-glycoprotein (Pgp), difficult regimens, and drug interactions. Many of the factors increase pharmacokinetic variability or limit

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bioavailability, leading to a number of patients with suboptimal plasma drug concentrations. The presence of suboptimal drug concentrations increases the likelihood of drug resistance and treatment failure. To decrease the occurrence of PI-resistance, investigators are attempting to increase trough PI plasma concentrations. The PIs are metabolized by CYP3A. Although all PIs inhibit CYP3A to some degree, ritonavir is a very potent CYP3A inhibitor. Many investigators coadminister a subtherapeutic dose of ritonavir with other PIs, to increase the concentrations of the PI. This practice is called pharmacologic enhancement. The advantages of this approach include raising trough drug concentrations, decreasing interpatient variability, prolonging drug elimination half-life to allow less frequent dosing, and decreasing pill burden. The approved product Kaletra™ is a fixed combination of the PI lopinavir with a subtherapeutic dose of ritonavir. Lopinavir is potent HIV PI, with very low bioavailability due to CYP3A first-pass metabolism. Adding a small dose of ritonavir increases lopinavir plasma concentrations manyfold [10].

As the practice of PI pharmacologic enhancement continues, the challenge is selecting the appropriate dose and regimen of the PI and ritonavir. Different combinations lead to different changes in PI plasma AUC, C_{max}, and trough concentration. Most investigators try to maximize trough and minimize C_{max}, under the assumption that trough is associated with efficacy and C_{max} is associated with toxicity. An improved understanding of exposure-response relationships for specific drugs and drug classes will help in the selection of appropriate enhanced regimens.

Antibiotics

Antibiotics are used to treat a wide range of bacterial infections, ranging from otitis media and urinary tract infections to serious lower respiratory tract infections and bacteremia. The primary goal of treatment with an antibiotic is selection of a drug and dosing regimen that is active against the infecting micro-organism at the site of action. Thus, in addition to being active against the micro-organism, the drug and dosing regimen must provide adequate amounts of active drug for an adequate amount of time at the site of infection. There are a number of in vitro methods that allow one to determine concentrations of drug that should be effective. These sensitivity tests indicate the minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) for drug-organism pairs. Patient immune defense system is also an important factor. If an antibiotic inhibits the growth of an organism, but does not kill it, the patient's immune

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system must be able to eradicate the micro-organism in order to achieve a cure [11].

Information about the exposure-response relationship for many classes of antibiotics arose due to integration of in vitro sensitivity tests, in vivo measures of antibiotic efficacy and understanding of bacterial action and antimicrobial action [12]. As first described by Shah et al. [13], there are two groups of antibacterial drugs, based on their pattern of bactericidal activity. The first group of drugs exhibit concentration-dependent killing, where higher drug concentrations lead to a greater rate and extent of bactericidal activity. Drugs in this group include fluoroquinolones and aminoglycosides [14–16]. For these drugs, the ratio of plasma AUC to MIC (AUC/MIC) or plasma C_{max} to MIC (C_{max}/MIC) correlates with efficacy. For the second group of drugs, the rate and extent of bacterial kill depends on duration of exposure and the effect saturates at low multiples of the MIC. Drugs in this group include ß-lactams, vancomycin, clindamcin, and macrolides [13–16]. The parameter often used to predict efficacy for this group of drugs is the time above the MIC. Complicating these two patterns of bacterial killing is the postantibiotic effect (PAE), which is the time it takes an organism to recover from the effects of exposure to an antibiotic. The PAE is demonstrated in vitro by observing bacterial growth kinetics after removing the drug [17]. However, the length of an in vitro PAE does not predict the duration of the in vivo PAE [18, 19].

Knowledge of the general relationships discussed above is useful when determining appropriate doses to study in infected patients. One can select the dose for further study based on in vitro sensitivity data and pharmacokinetic data from uninfected volunteers. Such a practice allows dose selection to occur without exposing infected patients to suboptimal antibiotic concentrations that may encourage the growth of resistant organisms.

Preston et al. [20] used exposure-response information to help determine the appropriate dose of levofloxacin for Phase III trials. The specific objective of the study was to prospectively quantitate the relationship between levofloxacin plasma concentrations and successful clinical and microbiological outcomes and occurrence of adverse events. The study included 313 patients with bacterial infections of the respiratory tract, skin, or urinary tract. The levofloxacin dose and treatment duration varied, depending on the site of infection. Patients received at least three intravenous levofloxacin doses and then completed therapy with oral levofloxacin, if medically appropriate. The primary analysis for this study included the 134 patients with concentration-time data and an identified organism with a determined MIC.

The clinical and microbiological response rates were 95 and 96%, respectively. The investigators evaluated the relationship between a number

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of factors and response rates, using logistic regression. The factors in the analysis included organism, site of infection, MIC of the organism, and the derived pharmacokinetic parameters peak, trough, AUC, Peak/MIC, AUC/MIC, and Time>MIC. The final model for clinical outcome included Peak/MIC ratio and site of infection as the predictors of clinical success. The Peak/MIC ratio break point was 12.2. The clinical success rate for patients achieving a ratio of greater than 12.2 was 99.0%; the rate for patients with a ratio of 12.2 or less was 83.3%. The final model for microbiologic outcome included Peak/MIC ratio as the predictor of microbiologic success. The Peak/MIC ratio break point was 12.2. The microbiological success rates for patients achieving a ratio of greater than 12.2 was 100%; the rate for patients with a ratio of 12.2 or less was 80.8%. Although Peak/MIC ratio was the most important derived pharmacokinetic measure for success, AUC/MIC ratio also predicted clinical and microbiologic success. Peak/MIC ratio and AUC/MIC ratio had similar predictive power because the two parameters are highly correlated with one another.

Based on the results of this study, knowledge of patient factors that affect pharmacokinetics, and MIC information, it is possible to select a levofloxacin dose that offers a high probability of successful treatment. The high success rate in this study indicates that the doses used were selected based on a great deal of prior knowledge about the drug. However, the study does allow greater confidence for the doses used in Phase III studies.

Drusano et al. [21] demonstrated a method for selecting a Phase II/III dose of an antibiotic using human pharmacokinetic data and animal pharmacodynamic data. The test agent was evernimicin, the first member of a new class of oligosaccharide antibiotics active against gram positive organisms. The investigators proposed that rational dose-selection decisions can be made based on a mathematical model that uses four data sets: the distribution of MICs for relevant clinical isolates, the distribution of the pharmacokinetic parameter values in the population, the derived pharmacokinetic/pharmacodynamic (PK/PD) target developed from animal models of infection, and protein-binding characteristics of the test drug. The animal model used was a neutropenic murine thigh infection model. Based on the animal model, AUC/MIC was the best predictor of microbiologic efficacy. The investigators used Monte Carlo simulations to determine the probability of attaining the target AUC/MIC with two different evernimicin doses and three different organisms. These investigators thus demonstrate one way to determine antibiotic doses for clinical study, prior to exposing large numbers of infected humans to the drug.

Both of the above examples indicate that exposure-response evaluations can assist in the determination of appropriate antibiotic doses for study in infected patients. In some cases the methods involve complex mathematical manipulations. The doses selected by these methods require confirmation in

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clinical efficacy and safety studies. Although the methods are complex and require further confirmation, they are of particular value in settings where suboptimal drug concentrations may lead to bacterial resistance. Also, the methods may decrease the time needed to identify a safe and effective dose.

Stroke and Cerebrovascular Diseases

Stroke and associated cerebrovascular disorders are a major public health concern and a leading cause of death in the United States and other countries [22]. Stroke is indicated by an abrupt manifestation of neurologic deficits secondary to an ischemic or hemorrhagic insult to a region of the brain. There are various candidate drugs for acute stroke, such as antithrombotic agents, anticoagulants, thrombolytic agents, and neuroprotectants. Thrombolytic agents, such as tissue plasminogen activator and streptokinase, are used in the management of thrombotic or nonhemorrhagic strokes. Neuroprotective drugs are designed to limit tissue damage and injury in the case of an infarct or hemorrhagic stroke.

Because stroke is a major cause of mortality and morbidity, much effort focuses on the development of drugs to limit brain damage. Approaches to the design of stroke trials and development of drugs for stroke benefit from the use of clinical pharmacology principles, such as appropriate dose selection, robust study designs, control of confounding factors, and selection of optimal endpoints. The application of clinical pharmacology principles helps provide therapeutic agents with better benefit-risk ratios and helps identify failures as early as possible [23].

Use of appropriate preclinical animal models for stroke is important in order to obtain early information regarding the pharmacological activity of the drug. The appropriate use of exposure-response relationships in preclinical drug development helps provide information that may be difficult to obtain in human subjects. For example, the neuroprotective effect of a novel, high-affinity serotonin (5-HT1A) agonist, BAY X3702, was tested in a rat model of acute subdural hematoma (ASDH) using different doses of the drug. The ischemic brain damage at four hours after ASDH was assessed for each dose group and was significantly smaller for the drugtreated group compared to the placebo-treated ASDH group. The results from this preclinical model gave a preliminary indication that this novel, high-affinity 5-HT1A agonist may have neuroprotective properties [24].

The importance of clinical pharmacology in preclinical development of drugs for this indication is further illustrated using an example of an antithrombotic agent that is a selective inhibitor of Factor Xa [25]. The

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progression of this candidate drug to Phase I studies was facilitated by useful PK/PD information obtained in preclinical studies. A thrombosis model in the dog was used to establish a PK/PD relationship for this drug; the biomarker was time to artery occlusion. Based on this study, an IC_{70} value of 250 ng/mL was estimated for the dog. In addition, in vitro data suggested that the pharmacodynamic response in humans was 2.5 times more sensitive than the response in the dog; therefore, the predicted IC_{70} in humans was 100 ng/mL. This information, in combination with that obtained from allometric scaling methods (to obtain estimates of pharmacokinetic parameters in humans), was used to select doses for the first Phase I study by targeting steady-state concentrations in the range of 100 ng/mL. This example emphasizes the importance of the appropriate use of exposure-response assessments in preclinical stages of drug development, because this can help develop rational dose selection in first-time-in-man studies.

Historically, Phase I studies conducted in healthy volunteers provide early information related to the safety, tolerability, and pharmacokinetics of a drug candidate. However, Phase I studies of drugs to treat stroke can also provide useful pharmacodynamic data to address proof of therapeutic concept. This type of information can generally be obtained fairly quickly and effectively in healthy volunteers. For example, for an antiplatelet agent, RGD 891 [25] information derived from exposure-response relationships established in Phase I studies was used to simulate optimal dosing regimens for Phase II studies. The pharmacodynamic response (% inhibition of platelet aggregation) observed in the actual Phase II studies in patients was similar to that observed in the Phase I studies. Exposure-response relationships established in the Phase I setting must be confirmed and further explored in Phase II studies in patients. An exposure-response database such as the one built for this antiplatelet agent can guide the design and dosing regimens for larger Phase III studies. Although, there may be some problems extrapolating from healthy volunteers to stroke patients, this approach is less problematic than extrapolating from experimental animal or in vitro studies.

Migraine

Migraine is one of the most common incapacitating headaches, and it afflicts approximately 23 million adults in the United States, with a 15% prevalence rate [26]. Most migraine patients suffer between one and six attacks a month and the duration of pain for each attack lasts between 4 and 72 hours. Care of migraine patients includes terminating migraine headache, preventing attacks, and improving quality of life. Some of the

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preventive agents used include beta-adrenergic blockers, calcium channel blockers, tricyclic antidepressants, anticonvulsant medications, and serotonin antagonists. Effective agents for treatment of acute migraine attacks include simple or combination analgesics, nonsteroidal anti-inflammatory drugs, ergot derivatives, selective serotonin agonists, and antiemetics. Most existing treatments are about 50–70% effective at two hours after administration of the drug. The placebo response is about 20–35% [26, 27].

The most recent approach to treatment of migraine headaches is the use of potent serotonin 5-HT1B/1D receptor agonists, which are collectively classified as triptans. Triptans are believed to exert their action by binding to serotonin receptors in the brain, where they induce vasoconstriction of extracerebral blood vessels and also reduce neurogenic inflammation. Sumatriptan was the first of these compounds developed that offered considerably improved efficacy and tolerability over the ergot-alkaloids. At present there are at least three other triptans available on the market that have similar or improved pharmacokinetic properties or efficacy and tolerability profiles compared to sumatriptin [28].

New approaches to trial design include using modeling and simulation strategies to address trial design questions. For example, during the development of a new triptan, the amount of useful information about the drug class, the disease, and the patient population is high. Information from preclinical animal models and mechanism of action are also available. Because the amount of information available in this case is large, few assumptions are needed to construct the models needed for trial simulations, and the uncertainty in the model predictions is usually low. Thus, the use of computer-assisted trial designs can help shorten and focus the development of the antimigraine triptan.

For the development of a new triptan, the objectives of modeling and simulations include the selection of the appropriate dose for further development. In the example discussed below, data from a Phase II dose-ranging study were used to develop a dose-response model for the triptan under development. The efficacy assessment measured headache severity on a 4-point scale (0=None, 1=Mild, 2=Moderate, 3=Severe). The pharmacodynamic endpoint used was the percent of patients who experienced headache relief (score of 0 or 1 at two hours).

A logistic regression model for pain relief was used to model the pain relief data and to construct a dose-response model for the triptan under consideration [29]. For this example, the modeling exercise was helpful in determining the median dose to achieve a target (for example 70%) pain relief, identifying two appropriate doses for further study (assuming that the tolerability profile at both doses was favorable) and determining the placebo response was approximately 40%.

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Information obtained from this type of modeling effort may be put to further use in simulations of the larger, conclusive Phase III trials prior to actually conducting the trial. Because simulations reflect uncertainty in model parameter values, they are useful in evaluating the distribution of model-predicted dose-response relationships. Simulations also allow calculation of the power to detect difference from placebo as a function of dose and sample size.

Gastric Acid-Related Disorders

Gastric acid-related disorders include heartburn, gastric and duodenal ulcers, symptomatic gastroesophageal reflux disease (GERD), erosive esophagitis, and pathological hypersecretory conditions such as Zollinger-Ellison syndrome. The conventional treatment for these acid-related disorders is the suppression of gastric acid secretion by H_2 blockers and proton pump inhibitors (PPIs). PPIs are currently the drugs of choice in the management of acid-related disorders. The use of antisecretory agents in combination with antibiotics is beneficial in the healing of H-pylori related peptic ulcers. The approved H_2 blockers in the United States include cimetidine, ranitidine, famotidine, and nizatidine. Approved PPIs include omeprazole, esomeprazole (enantiomer of omeprazole), pantoprazole, lansoprazole, and rabeprazole.

H_2 blockers principally act via competitive inhibition of H_2 receptors located on the parietal cells of the stomach. PPIs suppress gastric acid secretion by irreversibly inhibiting the gastric H^+/K^+ ATPase enzyme system at the secretory surface of the gastric parietal cell, thus blocking the final step of acid production. Both H_2 blockers and PPIs cause dose-related suppression of basal gastric acid secretion. However, the two classes of drugs differ markedly in their pharmacodynamic profiles. The antisecretory effect of H_2 blockers has a rapid onset and a relatively short duration. On the other hand, although PPIs generally have short plasma elimination halflives of about 1–2 hours, the antisecretory effect lasts for up to 3–5 days after drug administration [30]. The prolonged effect of PPIs is attributed to their mechanism of action, which involves irreversible inhibition of the proton pump. The rate-limiting step in the antisecretory action of PPIs is the turnover of the proton pump, which is reported to have a half-life of about 50 hours.

Studies in healthy volunteers can provide a preliminary evaluation of the potential efficacy of antisecretory agents and also dose-response information. Administration of pentagastrin or peptone meal provides acid stimulation in these studies. Thus, early Phase I studies designed to characterize the pharmacokinetics of the drug product can evaluate the

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pharmacodynamic effect as well. Pharmacodynamic biomarkers such as median 24-hour pH, % time gastric pH >3, and % time gastric pH >4 are common efficacy biomarkers for antisecretory agents. Use of these biomarkers has arisen from studies that utilized meta-analyses to determine the degree and duration of acid inhibition necessary for optimal healing of various acid-related disorders. The findings suggest that gastric pH has to be elevated above 3.0 for about 16–18 hours a day for treatment of duodenal ulcer, while gastric pH needs to be elevated above 4.0 for 16–18 hours a day for treatment of esophagitis. However, the clinical relevance of the above biomarkers is not established. Thus, if favorable data are obtained in healthy volunteers, then similar studies are carried out in patients to further characterize the gastric acid antisecretory effect. Subsequently, full clinical efficacy and safety studies can be initiated with clinical endpoints as the outcome (e.g., % of patients healed in active duodenal ulcer trial).

There is extensive literature that describes exposure-response relationships for H_2 blockers. In general, a direct correlation appears to exist between plasma concentrations of H_2 blockers and the acid inhibitory activity, which may be attributed to the competitive nature of drug-receptor interaction associated with H_2 blockers [31]. Exposure-response analyses relying on the sigmoid E_{max} model have been successful in predicting the time course of acid inhibitory activity for H_2 blockers [32].

Apparent exposure-response relationships are reported for most PPIs [31–35]. Katashima et al. [33] analyzed the relationship between plasma concentrations and the inhibitory effects of the PPIs omeprazole, lansoprazole, and pantoprazole on gastric acid secretion in healthy human subjects using a model that assumed a linear relationship between the fraction of inactive gastric proton pumps and the acid inhibitory effect. The authors concluded that the potency of the acid inhibitory activity of pantoprazole was weaker than that of omeprazole and lansoprazole, but the apparent recovery half-life of pantoprazole (45.9 hours) was slower than that of either omeprazole (27.5 hours) or lansoprazole (12.9 hours). It is noteworthy that while the model reasonably predicted the gastric acid inhibitory effects of studied PPIs, it may not have an actual mechanistic basis. More recently, Perron et al. [34] analyzed the exposure-response relationship for pantoprazole (10–80mg, IV & oral) in healthy human subjects using an indirect response model. The model reasonably described the time course of acid secretion at all studied doses. The authors concluded that maximum acid inhibition was related to the extent of exposure to pantoprazole. In addition, the time to maximum acid inhibition decreased with higher doses. Further work is needed in the area of exposure-response modeling of PPIs to fully characterize the time course of gastric acid

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inhibition exerted by PPIs. More importantly, further investigation is needed to explore the nature of the relationship between gastric acid inhibition and clinical efficacy in acid-related gastrointestinal disorders.

Pharmacodynamic data on antisecretory activity are useful in special populations and other situations in which clinical efficacy trials are not feasible. For example, measurement of antisecretory activity in pediatric patients is feasible and can be used in lieu of large clinical studies with efficacy endpoints. Pharmacodynamic data on antisecretory activity can also be obtained in special populations such as hepatic and renal impairment patients. The need for dosage adjustment in these special populations can be made by taking into account both pharmacokinetics and pharmacodynamcis. For many other disease states, the need for dosage adjustments in special populations are made based on pharmacokinetic data alone.

Two key clinical pharmacology issues arise with antisecretory treatment. The first issue is the potential effect of these agents on the absorption of coadministered drugs. Because these drugs markedly elevate the pH in the stomach, they may affect the pharmacokinetics of a coadministered drug with pH-dependent absorption or a modified-release drug product with pH-dependent drug release. For example, in normal subjects, coadministration of rabeprazole 20 mg once daily resulted in an approximately 30% decrease in the bioavailability of ketoconazole and increases in digoxin AUC and C_{max} of 19% and 29%, respectively [36]. Consequently, one may need to alter the time of drug administration or adjust the dose of the coadministered drug. The second issue is the effect of CYP2C19 phenotype on pharmacokinetics. Omeprazole, lansoprazole, pantoprazole, and esomeprazole are metabolized by CYP2C19, an enzyme that exhibits genetic polymorphism; approximately 3% of Caucasians and 17–23% of Asians are poor metabolizers. One can use exposure-response information to determine the need for dosage adjustment in these patients.

SPECIAL CLINICAL PHARMACOLOGY ISSUES FOR SPECIFIC DRUG CLASSES

Neuromuscular Blocking Agents

Neuromuscular blocking agents are used as adjuncts to general anesthesia to facilitate tracheal intubation and to provide skeletal muscle relaxation during surgical procedures. Rocuronium, vecuronium, pancuronium, and cisatricurium are some of the nondepolarizing neuromuscular blocking agents approved in the United States. These agents act by competing for

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cholinergic receptors at the motor end plate. Acetylcholinesterase inhibitors such as neostigmine, edrophonium, and pyridostigmine reverse the neuromuscular blockade by inhibiting the acetylcholine antagonism.

The exposure-response evaluation of neuromuscular blocking agents is aided by a quantifiable response endpoint. The response endpoint commonly used for evaluation of neuromuscular agents is mechanical response to train-of-four (TOF) stimulation measured at the adductor pollicis. There is an established and accepted methodology for administration of the stimulus to adductor pollicis and quantification of the response. Supramaximal square-wave TOF stimuli of 0.1–0.2 milliseconds duration are administered at 0.1–2 Hz every 12–20 seconds to the right ulnar nerve via surface electrodes placed at the wrist. The evoked tension of thumb adduction is measured with a calibrated transducer. Depression of the twitch response to the first stimulation in the TOF (T1), expressed as a percentage of the baseline value obtained prior to the administration of the drug, is used as a measure of the neuromuscular block. The relationship between plasma concentrations and neuromuscular block correlate consistently using the Sigmoid E_{max} model.

Exposure-response relationships for neuromuscular blocking agents have been successfully used to compare the features of a new drug relative to other drugs, to assess the contribution of a metabolite to the activity of a drug, and to assess the differences in special populations for potential dosage adjustments. For example, data obtained after separate administration of rapacuronium bromide and its 3-hydroxy metabolite showed that the metabolite has slower onset and higher potency (smaller EC_{50} value) than rapacuronium bromide [37]. Such data obtained in early Phase I trials can aid in the selection of the compound (parent or active metabolite) for further development. Such data can also be used to compare a product under development with products currently in use. Finally, evaluation of exposure-response data for cisatricurium indicated that the onset of effect may be marginally delayed, but otherwise there are no distinguishable differences in the effects observed in elderly patients compared to young adult patients [38].

Cancer Chemoprevention

Cancer chemoprevention refers to the inhibition or reversal of carcinogenesis using appropriate pharmacologically active agents to block the development of cancers in human beings. The goals of cancer chemoprevention are inhibition of carcinogens, logical intervention for persons at genetic risk for cancer, treatment of precancerous lesions, and

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confirmation and translation of leads from dietary epidemiology into intervention strategies [39].

The development and the evaluation of cancer chemoprevention strategies involve the use of a wide range of biomarkers. The term "biomarker" refers to internal indicators of exposure (biomarkers of exposure), indicators of adverse effect or desired effect (biomarkers of effect) or indicators of an intrinsic or acquired susceptibility to disease (biomarkers of susceptibility). Biomarkers help define exposure and disease status and may help identify possible interactions between risk factors and disease occurrence. A biomarker needs to be validated and its distribution in large populations described before it can be used reliably in clinical research. In chemoprevention, an exposure biomarker is a biologic substance that reflects endogenous or exogenous exposure to carcinogenic risk factors; this biomarker may be predictive of the incidence or outcome of disease. Exposure biomarkers may be used for assessment of exposure to external carcinogens such as **DNA** or protein adducts or for assessment of harmful endogenous agents such as abnormal hormonal levels [40]. A biomarker of intermediate effect is an indicator of the development of carcinogenic change (short of invasive cancer) in a patient. Examples of intermediate-effect biomarkers include: (1) adenomas for colorectal cancer—in chemoprevention trials for colorectal cancer, adenomatous polyps are used as biomarkers of risk; (2) the degree of mammary density as a proportion of the breast is associated with increased risk of breast cancer; and (3) tests for p53 mutations may indicate long-term changes for liver cancers [40]. A biomarker of susceptibility is an indicator of the ability of a patient to respond to the challenge of a carcinogenic agent. Biomarkers of susceptibility can help select high risk patient populations. For example, patients diagnosed with one type of cancer are at increased risk of a second primary cancer. Individuals in families with a genetic history of cancer may be more susceptible [40].

The development of the nonsteroidal anti-inflammatory agent sulindac as a chemopreventive drug used exposure-response assessments based on a biomarker, the inhibition of cyclooxeganse 2 (Cox-2), and enhancement of apoptosis [41]. Another example is the development of the R-isomer of flurbiprofen [42], which works in animal models as an antiproliferative agent against colon polyps, colonocytes, and adenocarcinomas, without the gastrointestinal toxicity of the S-isomer or the racemate. Although these biomarkers are not validated as surrogate endpoints, they may be used during drug development to help assess activity of compounds to prevent cancers. However, efficacy studies are needed to confirm the utility of the compounds.

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Antihypertensive Agents

Exposure-response information plays an important role in the development of drugs for the treatment of many cardiovascular illnesses, including hypertension. The surrogate markers measured as response for antihypertensive drugs include changes in blood pressure. Exposure-response data are usually collected in Phase II trials that are double-blind, randomized, placebo-controlled, and parallel-group in design. In the development of antihypertensive drugs such as the angiotensin-converting enzyme (ACE) inhibitors or beta blockers, it is important that exposure-response information be obtained across several orders of magnitude of doses in order to be able to determine the optimum dose for patients. In October 2000, the FDA convened an advisory committee meeting to discuss the importance of obtaining appropriate dose-response information during antihypertensive drug development. The committee concluded that elucidating the full range of dose-response relationships for antihypertensive drugs does not constitute an undue burden on investigators, and may help avoid the conduct of trials and experiments that do not contribute to the total knowledge of the appropriate exposure-response relationship.

Inhalation Drugs for Pulmonary Indications

Inhalation drugs used for pulmonary indications, such as asthma, present challenging exposure-response issues. It is presumed that the site of action is the local airway, so systemic exposure does not represent exposure at the site of action. Thus, systemic exposure does not predict clinical efficacy or local safety in the respiratory tract. There are some tools for assessing the extent of delivery to the lung. One common tool is scintigraphy. Systemic exposure is a possible tool when the drug has low oral bioavailability and high pulmonary bioavailability. However, these methods do not offer definitive proof of delivery to the relevant area of the lung. Thus, one must conduct a pharmacodynamic study to determine relevant doses for further study. The pharmacodynamic endpoints vary depending on the class of drugs. There are a number of direct measures of action for bronchodilators, including serial spirometry, protection against bronchoprovacation, and peak flow-rate assessments. There are no definitive direct measures of action for inhaled corticosteroids, but indirect measures include exacerbation rates, rescue use, and protection against bronchoprovocation.

In addition to endpoint issues, it is important to consider drug-device-patient interactions for orally inhaled drugs. Different devices (metered dose inhalers, dry powder inhalers, nebulizers) provide different patterns of deposition in the lung. Particle size also affects where the drug deposits, from the upper airway to the lower airway, or even being exhaled without

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deposition. It is also important to consider the effect of study population. One study indicated that following oral inhalation of fluticasone propionate, plasma concentrations were more than twice as high in normal volunteers compared to asthmatic patients [43].

Acute Pain—The Dental Pain Model

There are a number of situations in which patients experience acute pain. The post third molar extraction dental pain model is a useful model for the study of analgesia of acute pain. As described by Averbuch and Katzper [44], the model is relatively easy to study and there are few confounding factors. The dental pain studies are conducted in subjects scheduled to have their third molars removed. To be included in analysis, the subjects must experience moderate to severe pain following the extraction procedure. The study drug is administered after the pain assessment. Subjects can receive a local anesthetic, intravenous sedative agents, or antianxiety agents during the surgery; however, subjects cannot receive any analgesic for 24 hours prior to study. The efficacy endpoints include pain intensity score measured by a 4-point categorical scale (from 0=none to 3=severe) and pain relief score measured by a 5-point categorical scale (from 0=no relief to 4=complete relief). The scores are determined beginning just prior to drug administration and at various times until six hours postdose. Rescue analgesia medication is allowed, but subjects are excluded from further pain measurements afterwards. A measure of efficacy is the pain intensity difference (PID). The PID is calculated by subtracting the pain intensity at a specific assessment time from the baseline score. Positive values indicate a lessening of the patient's pain, while a negative value indicates increasing pain. One problem pointed out by Averbuch and Katzper is that patients who begin with severe pain can achieve a greater reduction in pain than patients who begin with moderate pain. One can stratify patients by baseline pain severity for statistical analysis. Investigators can use the dental pain model to compare two or more different drugs, to compare a new dug to placebo, or to evaluate several different doses of one drug. Although the dental pain model is simple and well defined, it is not clear how well the model represents all acute pain situations.

Immunosuppressive Agents

Immunosuppressive agents are used to prevent rejection of transplanted organs. Solid organ transplant recipients usually receive at least three antirejection agents, making it difficult to determine the contribution of a particular agent. The endpoint for evaluating these agents is occurrence of organ rejection. In many cases, the symptoms of rejection are similar to

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adverse effects of some of the drugs patients receive. Thus, it is often necessary to confirm acute rejection with a biopsy. The factors listed above complicate the evaluation of exposure-response relationships for immunosuppressive agents. However, because the transplant community appreciates the importance of exposure-response relationships for the safety and efficacy of immunosuppressive agents, studies of these relationships are common.

Van Gelder, et al. [45] evaluated the relationship between exposure and response for kidney transplant recipients receiving mycophenolate mofetil (MMF). Mycophenolate mofetil is a prodrug for the active moiety mycophenolic acid (MPA). The investigators randomized 154 adult recipients of kidney transplants to receive MMF treatment targeted at three predefined MPA AUC values (16.1, 32.2, and 60.6 μg*hr/mL). During the first six months after transplantation, investigators collected plasma samples for nine AUC evaluations. The primary endpoint of this six-month study was occurrence of biopsy-proven rejection. The analysis indicated that MPA predose concentration and MPA AUC are significantly related to the incidence of biopsy-proven rejection, and MMF dose is significantly related to the incidence of adverse events.

Although the study described above indicates it is possible to determine an exposure-response relationship for immunosuppressive agents, using the information to select a dose for patients is not simple. Most of the oral immunosuppressive agents have high inter- or intrapatient pharmacokinetic variability. Also, because the transplanted organ may participate in elimination of the drug, the pharmacokinetics of the drug may vary based on the time post transplantation. The exposure-response relationship may vary depending on doses of the other immunosuppressive agents in the regimen. For these reasons, transplant centers use therapeutic drug monitoring for some agents, including cyclosporine and tacrolimus. However, there is still debate regarding the appropriate exposure measure for therapeutic drug monitoring—minimum plasma concentration, full AUC, or limited sampling AUC.

Opioid Analgesic Agents

Opoid analgesic agents are used for the treatment of pain. Many of the old opioid drugs are being reformulated into novel dosage forms for better pain control and increased convenience. Routinely, studies characterizing the pharmacokinetics of the drug and drug product are conducted in healthy volunteers, to allow selection of a product with desired delivery properties. For opioids, whether new or a reformulation, Phase I studies present a challenge because healthy volunteers may not be able to tolerate the opioid effects, especially at high doses. Conducting these studies in patients,

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although an option, is impractical. A way to get around this problem is to provide the volunteers naltrexone blockade. Naltrexone, an opioid antagonist, may block the opioid effects without significantly affecting the pharmacokinetics of the drug of interest. Bashaw et al. [46] showed that the differences in morphine bioavailability were minimal when 60 mg controlled-release morphine sulfate was administered with and without naltrexone pretreatment. For other opioids, it may be worthwhile to conduct a pharmacokinetic study first with and without naltrexone pretreatment before such an approach is routinely adopted in other pharmacokinetic studies.

Lipid-Lowering Agents

Atorvastatin, cerivastatin, lovastatin, and simvastatin are HMG-CoA reductase inhibitors, a class of lipid-lowering compounds that reduce cholesterol biosynthesis. These drugs are characterized by low (5%–60%) and variable bioavailability attributed to extensive first-pass metabolism. Because the CYP3A enzyme mediates metabolism of all four drugs, the potential for significant drug-drug interactions when coadministered with CYP3A inhibitors is high. As such, appropriate metabolism, bioavailability, and drug interaction studies need to be conducted early during the development of a drug belonging to this class. The safety of the drug at doses comparable to the exposures seen in drug interaction studies can then be studied in patient populations in safety studies to make an informed decision regarding the safety of the drug in those situations.

Conclusions

As indicated in the introduction to this chapter, for most drug classes the goals of clinical pharmacology and exposure-response evaluations are the same—to understand the relationship between exposure and response and determine factors that may alter exposure and response. However, the utility of clinical pharmacology information throughout the various stages of drug development differs among drug classes.

The initial sources of information that contribute to the exposureresponse evaluation differ by drug class. Prior to human studies, in vitro studies for anti-HIV drugs and antibiotics provide estimates of target plasma concentrations for efficacy. Animal models provide an early evaluation of potential efficacy for some drug classes, including antibiotics and drugs to treat stroke and migraine. Although studies in healthy volunteers usually provide pharmacokinetic and safety information, the studies can provide activity and efficacy information for some drug classes, including drugs to treat stroke and gastric acid-related disorders. However,

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there are many drug classes that require actual patients for evaluation of drug activity, such as anti-HIV drugs, antibiotics, and drugs to treat migraine. Irrespective of whether efficacy and activity information can be obtained in healthy volunteers, the true or final assessment of safety and efficacy for any drug can only be conducted in target patient population.

A number of drug classes present clinical pharmacology challenges. Although drug interactions are possible with many classes of drugs, metabolism-based interactions are a particular problem with anti-HIV drugs and HMG-CoA reductase inhibitors. Due to their effects on gastric acid, anti-secretory agents can interact with drugs that have pH-dependent absorption. It is difficult to determine exposure-response relationships for inhaled drugs, because systemic concentrations are often quite low and may not correlate with concentrations at the site of action. In situations where patients almost always receive multiple drugs for the same indication (HIV, organ transplantation), it is difficult to determine the contribution of individual drugs to response. Finally, biomarkers for use in exposure-response evaluations are not available for some drug classes.

In closing, the information in this chapter provides examples that support the clinical pharmacology principles discussed in other chapters in this book. Specific characteristics of the relevant disease state, patient population, drug class, and drug product influence the utility of various clinical pharmacology evaluations for a drug.

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Issues in Bioequivalence and Development of Generic Drug Products

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INTRODUCTION

The topic of bioequivalence evaluation of generic drug products seems simple but stimulates intense controversy and misunderstanding. For example, one often hears members of the public and medical experts alike stating various opinions on the unacceptability of approved generic drug products based on misconceptions about the determination of therapeutic equivalence of these products to the approved reference. These misconceptions include the belief that the Food and Drug Administration (FDA) approves generic products that have mean differences from the reference product of 20–25% and that generic products can differ from each other by as much as 45%. In addition, some incorrectly assume that, since most bioequivalence testing is carried out in normal volunteers, it does not adequately reflect bioequivalence and therefore therapeutic equivalence in patients. When the current bioequivalence methods and statistical criteria

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are clearly understood it becomes apparent that these methods provide a strict and robust system that provides assurance of therapeutic equivalence. In this chapter we will discuss the rationale and methods utilized for the demonstration of bioequivalence for regulatory purposes in the United States. In addition, we will touch on some controversial issues and difficulties in demonstrating bioequivalence for certain classes of drug products.

Bioavailability is the rate and extent of drug appearance at the site of activity. It reflects both drug substance disposition properties as well as formulation-related effects. In contrast, bioequivalence involves the comparison of rate and extent of drug availability between two or more formulations containing the same drug substance. In other words, bioequivalence is a comparison of in vivo formulation performance. At first it might appear to be a simple matter to compare the performance of different formulations. In most cases, for comparison of formulation performance of systemically available drugs, the appearance of parent drug in the blood can be effectively used to discern the rate and extent of drug availability from different formulations. However, there are a number of drug products for which pharmacokinetic measures in blood are not appropriate for the demonstration of bioequivalence. These include those drug products that are applied to the site of activity to obtain a local therapeutic effect, i.e., the locally acting drug products. Topical products for the treatment of skin diseases, nasal spays for the treatment of allergic rhinitis, and inhalers for the treatment of asthma are examples of this type of product. For any of these products, differences in product performance cannot be adequately evaluated by attempting to measure the appearance of the drug in blood. Often the amount of drug absorbed into the blood is very small and difficult to measure and, more importantly, therapeutic effects are not related to the systemic absorption of the drug.

Most studies determining bioequivalence between generic products and the corresponding reference-listed drug products (commonly a brand-name product approved through the new drug approval process) are based on evaluation of blood concentration data in healthy subjects. It is true that drug pharmacokinetic profiles may differ between healthy subjects and particular types of patients. This is because some disease states affect different aspects of drug substance absorption, distribution, metabolism, and elimination. However, the effects of disease on relative formulation performance, i.e., release of the drug substance from the drug product, are rare. Bioequivalence studies are designed to measure and compare formulation performance between two drug products within the same individuals. It is expected that the relative difference in in vivo drug release between the two formulations will be the same whether the two formulations are tested in patients or normal subjects. Thus, generic and

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reference-listed drug products that are bioequivalent can be substituted for each other in patients because they will produce the same therapeutic effect(s) and have the same safety profile. This is illustrated by findings from a recent observational cohort study comparing effectiveness and safety in patients switched from brand-name warfarin sodium tablets to generic warfarin sodium tablets [1]. The generic product was approved based on standard bioequivalence studies in normal volunteers. The observational cohort study showed that the two products had no difference in clinical outcome measures.

Bioequivalence studies are also submitted to the FDA in certain situations for new drug products. For new drug products, bioequivalence documentation can be useful to establish links between (1) early and late clinical trial formulations; (2) formulations used in clinical trials and stability studies, if different; (3) clinical trial formulations and the to-be-marketed drug products; and (4) other appropriate comparisons. The same issues of bioequivalence study design, statistical analysis, and data interpretation apply to both new drug products and generic drug products.

FEDERAL REGULATIONS GOVERNING BIOEQUIVALENCE STUDIES OF GENERIC DRUG PRODUCTS

Title 21 of the Code of Federal Regulations (21 CFR) Part 320 contains the *Bioavailability and Bioequivalence Requirements* pertaining to registration of generic drug products in the United States. Part 320 consists of Subpart A, *General Provisions,* and Subpart B, *Procedures for Determining the Bioavailability and Bioequivalence of Drug Products.* Subpart A describes general provisions including definitions of bioavailability and bioequivalence. Subpart B states the basis for demonstrating in vivo bioavailability or bioequivalence and lists types of evidence to establish bioavailability or bioequivalence, in descending order of accuracy, sensitivity, and reproducibility. Subpart B also provides guidelines for the conduct and design of an in vivo bioavailability study and lists criteria for waiving evidence of in vivo bioequivalence (bio waivers). The bio waiver regulations apply to all parenteral solutions, including intraocular, intravenous, subcutaneous, intramuscular, intraarterial, intrathecal, intrasternal, and interperitoneal, but do not permit automatic waivers for all topical and nonsystemically absorbed oral dosage products [2]. In addition, biowaivers can be granted for ophthalmic, otic, topical, and oral solutions. Finally, biowaivers can be granted for a number of oral drug products approved before 1962 and formally evaluated in the late 1960s by a Congressionally mandated panel of scientific experts under the drug efficacy study implementation (DESI). The DESI panel formulated a list of pre-1962

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drugs that had demonstrated effectiveness and lacked bioequivalence problems [3]. For these DESI-effective drugs, the FDA waives in vivo studies provided that formulation and in vitro dissolution data are acceptable.

STATISTICAL EVALUATION OF BIOEQUIVALENCE DATA

Statistical evaluation of most bioequivalence studies is based on analysis of drug serum, plasma, or whole blood concentration data. The area under the plasma concentration vs. time curve (AUC) is used as an index of the extent of drug absorption. Generally, both AUC determined until the last quantifiable concentration sampled ($AUC_{0\text{-}t}$) and AUC extrapolated to infinity (AUC∞) are evaluated. Maximum postdose plasma concentration (C_{max}) is used as an index of the rate of drug absorption.

To statistically compare generic and innovator AUC and C_{max} data, the FDA uses the two one-sided tests statistical procedure, also referred to as the 90% confidence interval approach. The two one-sided tests procedure encompasses two questions [4]. Stated simply, the first test asks if the test (generic) product is significantly less bioavailable than the reference (usually brand-name) product. The second question asks if the reference product is significantly less bioavailable than the test product. A significant difference is defined as 20% at the alpha equals 0.05 level. Based on these statistical criteria, the mean test/reference ratio of the data is usually close to one. The criteria above may be restated to illustrate the rationale for the 0.80-1.25 (or 80%-125%) confidence interval criteria. In the first case illustrated above, test/reference=0.80 and in the second case (or bioequivalence limit) reference/test=0.80 (expressed by convention as test/reference=1.25, i.e., the reciprocal of 0.80). This may be stated in clinical terms as follows. If a patient is currently receiving a brand-name reference product and is switched to a generic product, the generic product should not deliver significantly less drug to the patient than the brand-name product; conversely, if a patient is currently receiving the generic product and is switched to the brand-name reference product the brand-name product should not deliver significantly less drug to the patient than the generic.

CURRENT METHODS AND CRITERIA FOR DOCUMENTING BIOEQUIVALENCE

The FDA Guidance for Industry, *Bioavailability and Bioequivalence Studies for Orally Administered Drug Products—General Considerations,* provides recommendations to firms planning to include bioavailability and bioequivalence information for orally administered drug products in regulatory submissions [5]. The guidance addresses how to meet the

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Bioavailability/Bioequivalence Requirements set forth in 21 CFR Part 320 as they apply to oral dosage forms. The guidance also applies to nonorally administered drug products where reliance on systemic exposure measures is suitable to document bioavailability/bioequivalence (e.g., transdermal systems, certain rectal, and nasal drug products.). The guidance is applicable to both generic products and new drug products.

There are several types of studies commonly used for demonstration of bioequivalence. The preferred study for most orally administered dosage forms is a two-way crossover, two-period, two-sequence single-dose study, under fasting conditions performed in normal healthy volunteers. In this design, each study subject receives each treatment, test and reference, in random order. Plasma or blood samples are collected for approximately three pharmacokinetic elimination half-lives for determination of the rate and extent of drug release from the dosage form and absorption by each subject. A washout period is scheduled between the two periods to allow the subjects to completely eliminate the drug absorbed from the first dose before administering of the second dose. Although this design is carried out for most orally absorbed drug products, it may become impractical for drugs with long pharmacokinetic half-lives, i.e., longer than 30 hours (e.g., amiodarone, clomiphene). In this case a single-dose parallel design may be used instead [6]. For drugs with very long half-lives, concentration sampling may be carried out for a period of time corresponding to two times the median T_{max} (time to C_{max}) for the product. For drugs that demonstrate low intrasubject variability in distribution and clearance, an AUC truncated at 72 hours may be used in place of $AUC_{0\text{-}t}$ or AUC4 [5]. An alternative study design that is recommended for modified-release products and for highly variable drug products is a replicate design [5]. In this design, each treatment is repeated in the same subject on two separate occasions. This is performed as either a partial (three-way) or full (four-way) replication of treatments.

Because food can influence the bioequivalence between test and reference products, the FDA recommends that applicants developing generic products (ANDA applicants) for oral administration conduct bioequivalence studies under fed conditions in addition to the fasting bioequivalence studies [7]. Fed bioequivalence studies should be conducted for all generic modified-release oral dosage forms because the bioavailability of these products is likely to be altered by coadministration with meals. For generic immediate-release oral dosage forms, the FDA recommends fed bioequivalence studies whenever the label of the reference-listed drug makes statements about the effect of food on the bioavailability of the drug product. Fed bioequivalence studies are not recommended for generic products if the label states that the product should be taken only on an empty stomach. Thus, the majority of regulatory submissions for generic drug products for oral administration

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will include at least two in vivo bioequivalence studies: one under fasting conditions and one under fed conditions.

By contrast, for new drug products, fed bioequivalence studies are rarely conducted. As previously stated, for new drug products, bioequivalence studies are conducted to compare to-be-marketed formulations with the clinical trial formulations and, in some circumstances, to compare new formulations with previously approved formulations. The FDA recommends that such bioequivalence studies for new drug products should be generally conducted in fasted subjects [7]. Applicants developing new drug products for oral administration usually conduct separate studies designed to directly compare drug bioavailability in fed and fasted subjects.

Fed bioequivalence studies are generally conducted using meal conditions expected to provide the greatest effects on formulation performance and gastrointestinal physiology such that systemic drug bioavailability is maximally effected. Typically, the drug is administered to subjects within 30 minutes of consuming a high-fat, high-calorie meal. The FDA recommends that these studies use a randomized, balanced, single-dose, two-treatment, two-period, two-sequence crossover design [5]. For a few drug products, such as mefloquine, the FDA recommends that applicants evaluate bioequivalence only under fed conditions because there are safety concerns associated with administration of the product on an empty stomach.

The FDA recommends that in vivo bioequivalence studies be conducted in individuals representative of the general population, taking into account age, sex, and race factors [5]. For example, if a drug product is to be used in both sexes, the sponsor should attempt to include similar proportions of males and females in the study; if the drug product is to be used predominantly in the elderly, the applicant should attempt to include as many subjects of 60 years of age or greater as possible. Restrictions on admission into the study should generally be based solely on safety considerations.

Bioequivalence studies should be conducted in the intended patient population when there are significant safety concerns associated with use in healthy subjects. For example, an antineoplastic drug intended for short-term therapy, such as etoposide, can be evaluated following a single dose either in cancer patients in remission or in patients under active treatment by sampling on the first day of a treatment cycle. As another example, for the medication clozapine, normal subjects may experience serious orthostatic hypotension with the first dose. Moreover, clozapine requires dose titration to achieve the maximum-tolerated, approved regimen, which is generally achieved using multiples of the highest approved strength. Thus, for clozapine, the most appropriate study design is a steady-state (multiple dose) crossover bioequivalence study in patients [8].

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TYPES OF EVIDENCE TO ESTABLISH BIOAVAILABILITY AND BIOEQUIVALENCE

General Considerations

Subpart B of the *Bioavailability and Bioequivalence Requirements* in 21 CFR Part 320 lists the following in vivo and in vitro approaches to determining bioequivalence in descending order of accuracy, sensitivity, and reproducibility [9]:

- In vivo measurement of active moiety or moieties in blood, plasma, or serum.
- In vivo measurement of the active moiety in urine.
- In vivo pharmacologic (pharmacodynamic) comparison.
- Well-controlled clinical trials.
- In vitro comparison.
- Any other approach deemed appropriate by FDA.

Figure 1 illustrates, for a model of oral dosage form performance, why the most sensitive approach is to measure the drug in biological fluids, such as blood, plasma, or serum. The active ingredient leaves the solid dosage form and dissolves in the gastrointestinal tract, and following absorption through the gut wall, appears in the systemic circulation. The step involving dissolution of the drug substance prior to absorption is the critical step, necessary for the absorption of the drug, that is determined by the formulation. Other steps illustrated in the diagram are patient- or subject-determined processes not directly related to formulation performance. Variability of the measured endpoint increases with each additional step in the process. Therefore, variability of clinical measures is quite high compared to blood concentration measures. Figure 2 shows that the blood concentration of a drug directly reflects the amount of drug delivered from the dosage form.

In situations where a drug cannot be reliably measured in blood, it may be appropriate to base bioequivalence evaluation on an in vivo test in humans in which an acute pharmacologic (pharmacodynamic) effect is measured as a function of time. Generally, the pharmacodynamic response plotted against the logarithm of dose appears as a sigmoidal curve, as shown in Fig. 3. It is assumed that, after absorption from the site of delivery, the drug or active metabolite is delivered to the site of activity and, through binding to a receptor or some other mechanism, elicits a quantifiable pharmacodynamic response. Since additional steps contribute to the observed pharmacodynamic response, a pharmacodynamic assay is not as sensitive to drug formulation performance as blood drug concentrations. In

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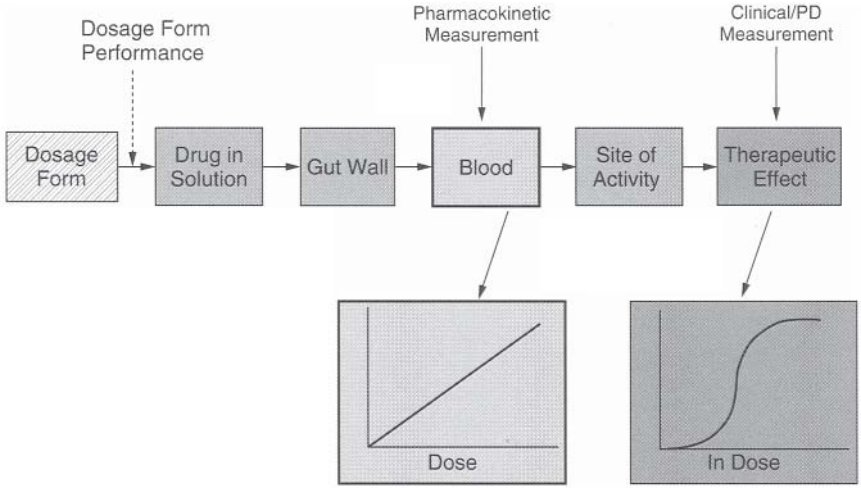

FIGURE 1 The most sensitive approach in evaluating bioequivalence of two formulations is to measure drug concentration in biological fluids, as illustrated in this diagram showing the relationship between dosage form performance and therapeutic response. Following oral dosing, the active ingredient leaves the solid dosage form, dissolves in the gastrointestinal tract, and, following absorption through the gut wall, appears in the systemic circulation. Formulation performance is the major factor determining the critical steps of dosage form disintegration and drug substance dissolution prior to absorption. All other steps following in vivo drug substance dissolution are patient-or subject-determined processes not directly related to formulation performance. The variability of the measured endpoint increases with each additional step in the process, such that variability of clinical measures is quite high compared to that of blood concentration measures. As a result, a pharmacodynamic or clinical approach is not as accurate, sensitive, and reproducible as an approach based on plasma concentrations.

developing a pharmacodynamic assay for bioequivalence evaluation, it is critical to select the correct dose. The dose should be in the range that produces a change in response, as shown in the midportion of the curve. In other words, the pharmacodynamic assay should be sensitive to small changes in dose. A dose that is too high will produce a minimal response at the plateau phase of the dose-response curve, such that even large differences in dose will show little or no change in pharmacodynamic effect. Depending on the type of response, a pharmacodynamic study can be conducted in healthy subjects. The pharmacodynamic response selected should directly reflect dosage form performance and availability at the site of activity but may not necessarily reflect therapeutic efficacy.

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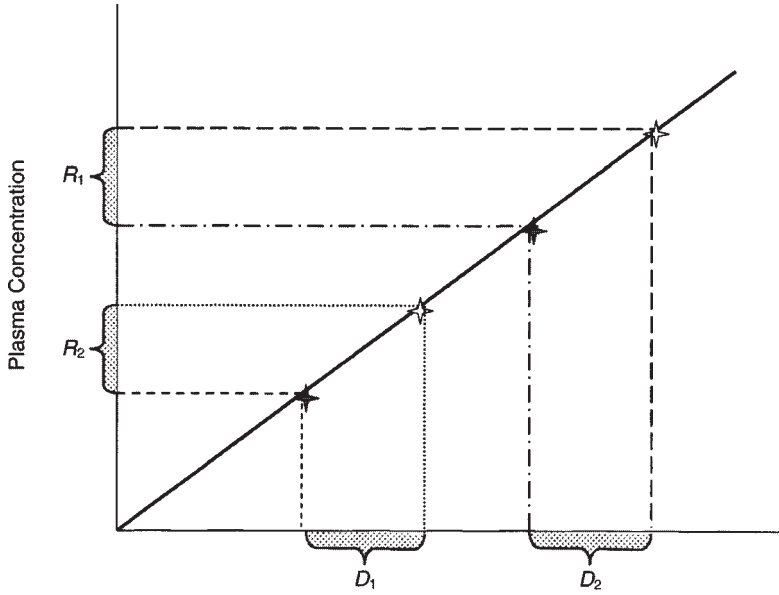

FIGURE 2 The blood concentration of a drug directly reflects the amount of drug delivered from the dosage form. The corresponding responses over a wide range of doses will be of adequate sensitivity to detect differences in bioavailability between two formulations. This is illustrated for two widely different doses, D_1 and D_2. Any differences in dosage form performance are reflected directly by changes in blood concentration (R_1 and R_2).

If it is not possible to develop reliable bioanalytical or pharmacodynamic assays, then it may be necessary to evaluate bioequivalence in a well-controlled trial with clinical endpoints. This type of bioequivalence study is conducted in patients and is based on evaluation of a therapeutic, i.e., clinical response. The clinical response follows a similar dose-response pattern to the pharmacodynamic response, as shown in Fig. 3. Thus, in designing bioequivalence studies with clinical endpoints, the same considerations for dose selection apply as for bioequivalence studies with pharmacodynamic endpoints. As with a pharmacodynamic study, the appropriate dose for a bioequivalence study with clinical endpoints should be on the linear rising portion of the dose-response curve, since a response in this range will be the most sensitive to changes in formulation performance. Due to high variability and the sometimes subjective nature of clinical evaluations, the clinical response is often not as sensitive to differences in drug formulation performance as a pharmacodynamic response. For these

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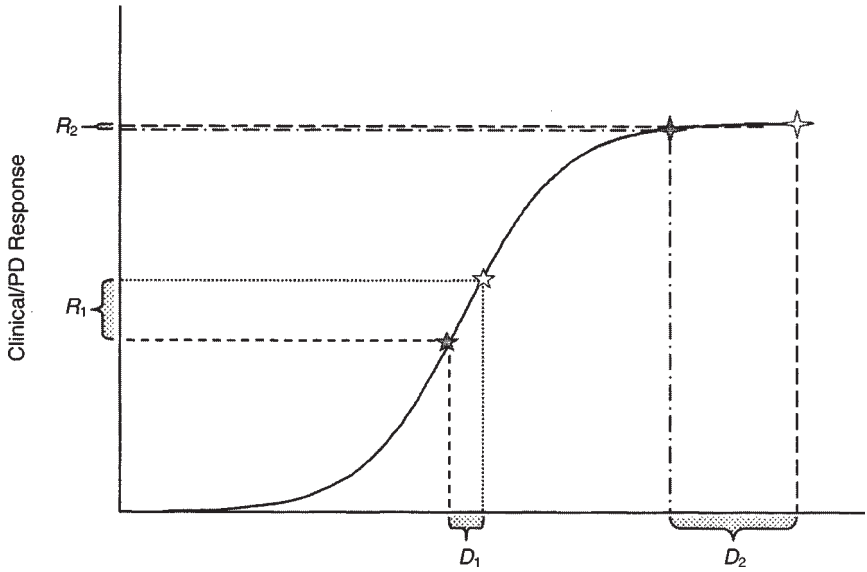

FIGURE 3 In evaluating bioequivalence in a study with pharmacodynamic or clinical endpoints, it is critical to select a dose that falls on the middle ascending portion of the sigmoidal dose—response curve. The most appropriate dose for a study based on pharmacodynamic or clinical endpoints should be in the range that produces a change in response *(R_1),* as shown in the midportion of the curve *(D_1)*. A dose that is too high will produce a minimal response at the plateau phase of the dose—response curve, such that even large differences in dose *(D_2)* will show little or no change in pharmacodynamic or clinical effect *(R_2)*. Thus, two formulations which are quite different may appear to be bioequivalent.

reasons, the clinical approach is the least accurate, sensitive, and reproducible of the in vivo approaches to determining bioequivalence.

Blood, Plasma, or Serum

Most bioequivalence studies submitted to the FDA are based on measuring drug concentrations in plasma. In certain cases, whole blood or serum may be more appropriate for analysis. Measurement of only the parent drug released from the dosage form, rather than a metabolite, is generally recommended because the concentration-time profile of the parent drug is more sensitive to formulation performance than a metabolite, which is more reflective of metabolite formation, distribution, and elimination [5]. Measurement of a metabolite may be preferred when parent drug

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concentrations are too low to permit reliable measurement. Metabolites formed by presystemic metabolism that contribute meaningfully to safety and efficacy are also measured in addition to the parent.

Urine

Urine measurements are not as sensitive as plasma measurements, but are necessary for some drugs such as orally administered potassium chloride [10], for which serum concentrations do not accurately reflect the amount of drug absorbed from the dosage form. Both cumulative amount of drug excreted (Ae) and maximum rate of urinary excretion *(R_{max})* are evaluated statistically in bioequivalence studies which rely on urine concentrations.

Studies of Pharmacologic (Pharmacodynamic) Effects

The FDA accepts pharmacodynamic effect methodology to approve generic topical corticosteroid drug products [11]. This approach is based on the ability of corticosteroids to produce blanching or vasoconstriction in the microvasculature of the skin. Since this property is presumed to relate to the amount of drug leaving the dosage form and entering the skin, the vasoconstriction assay has become the means for assessing bioavailability and bioequivalence of topical corticosteroids. In designing a bioequivalence study based on vasoconstriction, an applicant should first conduct a pilot study using the reference topical corticosteroid product to determine the dose-duration that will give the half-maximal response (ED_{50}). During the pivotal study, the test and reference products are applied to subjects' forearms for a dose-duration approximately equal to the ED_{50}. If the ED_{50} is estimated correctly in the pilot study, then the pivotal study will be adequately sensitive to differences in formulation performance. For bioequivalence analysis, 90% confidence intervals are determined for ratios of test and reference area-under-the-effect-curve (AUEC) data; these should fall within the range of 0.80-1.25.

Well-controlled Clinical Trials

Bioequivalence study designs with clinical endpoints are used with some topical products that are active at the site of application, such as tretinoin topical formulations. This approach is also used for some oral drug products that are not systemically absorbed, such as sucralfate tablets. Bioequivalence studies with clinical endpoints generally employ a randomized, blinded, balanced, parallel design. Studies compare the efficacy of the test product, innovator product, and placebo to determine if the two products containing active ingredient are bioequivalent. The placebo is

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included to assure that the two active treatments in the clinical trial actually are being studied at a dose that is pharmacologically and clinically active. Failure to assure that the treatments are clinically active in the trial would show that the trial has no sensitivity to differences in formulation performance, i.e., the response is on the flat bottom of the dose—response curve (Fig. 3). A generic equivalent of the innovator product should be able to demonstrate bioequivalence for selected clinical endpoint(s) that adequately reflect drug appearance at the site(s) of activity and therefore formulation performance. For example, for tretinoin topical cream formulations indicated for treatment of acne vulgaris, the endpoints relate to severity and number of lesions, whereas for sucralfate tablets, the clinical endpoint is duodenal ulcer healing at four weeks [6]. The test and reference clinical responses are considered bioequivalent if the 90% confidence interval for the differences in proportions between test and reference treatment is contained within the limits of -0.20 to 0.20.

In vitro Tests

With suitable justification, bioavailability and bioequivalence may be established by in vitro studies alone. This approach is also suitable for some types of locally acting products such as nasal solution aerosols/sprays, which produce effects on nasal sites of action without relying upon systemic exposure, and cholestyramine resins, which form nonabsorbable complexes with bile acids in the intestine. The FDA evaluates in vitro bioequivalence of nasal sprays and aerosols only for products with the same formulations within the spray device as the corresponding innovator products [12]. Therefore, the in vitro performance measures assess comparative performance of the devices used for administration. Test/reference ratios for dose/spray content uniformity, droplet/particle size distribution, spray pattern, and plume geometry measurements should be equivalent between the two products. For cholestyramine resins, the in vitro measures of bioequivalence are based on the rates of binding to bile acid salts [13]. The 90% confidence of the test/reference ratios of the equilibrium binding constants should fall within the limits of 0.80 to 1.25.

Waivers of in vivo Bioequivalence based on in vitro Testing

Under certain circumstances, product quality bioavailability and bioequivalence can be documented using in vitro approaches [9]. In vitro dissolution testing to document bioequivalence for nonbioproblem DESI drugs remains acceptable. In vitro dissolution characterization is encouraged for all product formulations investigated, including prototype formulations, particularly if in vivo absorption characteristics are being

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defined for the different product formulations. Such efforts may enable the establishment of an in vitro-in vivo correlation. When an in vitro-in vivo correlation is available [2], the in vitro test can serve as an indicator of how the product will perform in vivo.

DRUGS THAT ARE ALSO ENDOGENOUS SUBSTANCES

Bioequivalence studies of endogenous drug substances need special considerations. This is because for these substances there are measurable baseline concentrations in biological fluids, either because the product is manufactured in the body, such as levothyroxine or ursodiol, or is available from dietary sources, such as potassium chloride [14]. As previously stated, bioequivalence studies are conducted to compare formulation performance. With most drug products, the only source of the drug appearing in the blood is from the dosage form. With endogenous substances, there are two or more sources causing the substance to appear in blood. Adding complexity are feedback processes with substances like hormones, circadian rhythms, and influxes from the diet. Figure 4 shows that, following dosing with an endogenous substance, both release from the dosage form and body production contribute to blood levels.

Thus, in most cases, the FDA recommends baseline correction for endogenous substances. Measurement of the endogenous baseline depends on the characteristics of the endogenous substance. Often, a baseline is determined from one to three measurements taken before the drug products are given. Less often, sampling at regular intervals throughout the day for at least two days prior to dosing is performed. The baseline sampling should take place at several intervals to account for fluctuations due to circadian rhythms. Corrections should be subject- and period-specific. One important consideration in comparing generic and reference products is to give an adequate dose, because the plasma concentrations have to be high enough so that the substance can be accurately and reliably determined by the assay, after baseline correction. The objective is to discern any differences between a generic and reference product, without failing products that are almost identical.

Potassium chloride presents a special case. Serum measurements cannot be used for bioequivalence studies of potassium chloride products. Because homeostatic mechanisms maintain potassium concentrations in biological fluids within a narrow range, serum concentrations change minimally in response to a bolus dose. As shown in Fig. 5, the baseline is very high relative to any changes occurring after dosing. In fact, in pharmacokinetic studies of postassium chloride tablets, following an 80 mEq dose, serum potassium increases only about 5% relative to baseline [14]. Since virtually

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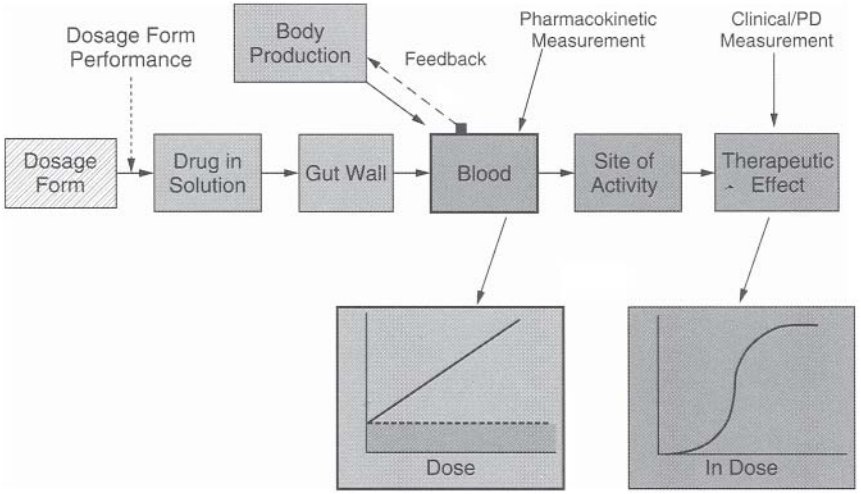

FIGURE 4 Two or more sources contribute to blood levels of a drug that is already present in the body as an endogenous substance. The drug that appears in the blood and throughout the body arises from body production in addition to release from the dosage form. With some endogenous substances, especially hormones, there can be a feedback process such that production and storage of the compound changes as blood or body concentrations change. When determining bioequivalence of formulations of these types of drugs, it may be necessary to use a baseline correction to account for the amount in blood that did not come from the formulation.

all of ingested potassium is excreted in urine, measuring urine output of potassium is an accurate means of comparing the potassium released from generic vs. reference formulations. The FDA recommends that, for potassium chloride bioequivalence studies, subjects ingest a standardized potassium diet for an equilibration period of several days before sampling takes place [10]. This practice helps achieve a relatively stable baseline before dosing starts.

COMPLEX DRUG SUBSTANCES

There are many drug substances that may fit into the category of "Complex Drug Substances." These include many proteins, peptides, botanicals, synthetic hormones, biotechnology products, and complex mixtures. For most of these drugs, the most difficult problem is to demonstrate

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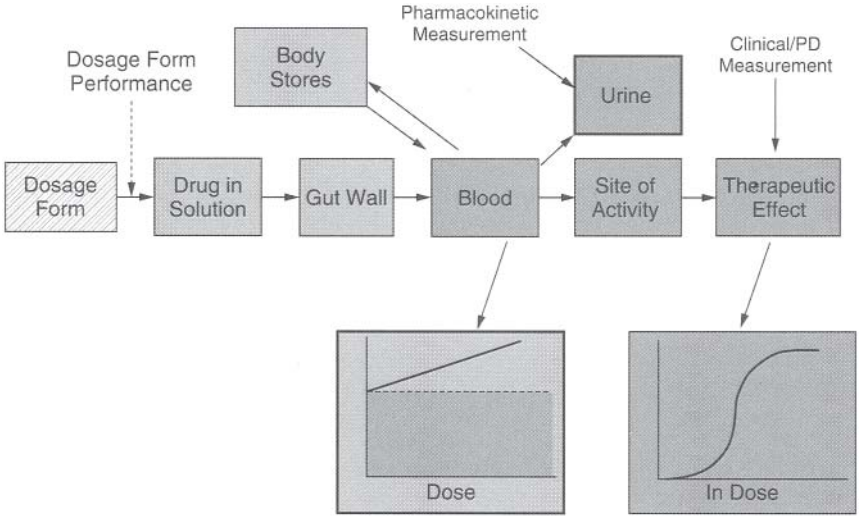

FIGURE 5 Unlike endogenous substances such as hormones which are synthesized by the body, endogenous potassium arises solely from dietary sources. The body transports potassium from place to place and excretes excess amounts primarily into the urine. Thus, a patient deficient in potassium will utilize supplemental potassium, whereas normal volunteers ingesting adequate levels of potassium will excrete virtually all, if any, excess. Because homeostatic mechanisms maintain blood potassium levels within a narrow range, there is very little change in blood levels following a potassium dose. This means that following a dose of potassium, a high percentage of the resulting potassium blood levels is due to the baseline that was already present before dosing. As a result, blood is not a good site for sampling for bioequivalence studies of oral dosage forms delivering potassium. Since most of an ingested dose is excreted in urine, bioequivalence is documented by measuring amounts of potassium excreted in urine. Urinary data must still be corrected for baseline, but this baseline represents a much smaller percentage of the total excreted.

pharmaceutical equivalence, i.e., that the drug substances are actually the same within each manufacturer's dosage form. In many cases, current technology is not sufficient to unequivocally characterize the drug substance in two different manufacturer's products or after a single manufacturer wishes to make pre or postapproval changes in manufacturing procedures. These challenges in drug substance characterization methods currently may stand in the way of the approval of generic products for many of these products containing complex drug substances.

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NARROW THERAPEUTIC INDEX DRUGS

There are no additional approval requirements for generic versions of narrow therapeutic index (NTI) drugs vs. non-NTI drugs. The FDA does not set specific standards based on therapeutic index [5, 15]. The bioequivalence criteria, using the 90% confidence interval approach, are quite strict; there is no need to apply stricter criteria for NTI drugs. The current FDA position is that any generic product may be switched with its corresponding reference-listed drug.

SUMMARY

Current bioequivalence methods in the United States are designed to provide assurance of therapeutic equivalence of all generic drug products to their innovator counterparts. The sole objective of bioequivalence testing is to measure and compare formulation performance between two or more pharmaceutically equivalent drug products. For generic drugs to be approved in the United States, they must be pharmaceutically equivalent and bioequivalent to be considered therapeutically equivalent and therefore approvable. In the United States, Part 320 of 21 CFR, the *Bioavailability and Bioequivalence Requirements,* states the basis for demonstrating in vivo bioequivalence, lists the types of evidence to establish bioequivalence (in descending order of accuracy, sensitivity, and reproducibility), and provides guidelines for the conduct and design of an in vivo bioavailability study. Through the years, the U.S. FDA has published Guidances for Industry which address how to meet the *Bioavailability and Bioequivalence Requirements* set forth in 21 CFR Part 320. The FDA updates these Guidances as the need arises to ensure that they reflect state-of-the art scientific thinking regarding the most accurate and sensitive methods available to demonstrate bioequivalence between two products. Consulting with panels of experts such as Advisory Committees, participating in meetings and workshops with Academia and Industry (both in the United States and abroad), and inviting public comment on draft guidances are among the mechanisms that the FDA employs to keep Guidance development current.

The FDA's current statistical criteria for determining acceptability of bioequivalence studies are designed to assure that the test product is not significantly less bioavailable than the reference (usually the innovator) product, and that the reference product is not significantly less bioavailable than the test product. The difference for each of these two tests cannot exceed 20%, with the result that the test/reference ratios of the bioequivalence measures must fall within the limits of 0.80 to 1.25. A

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generic product which does not meet these criteria is not approved. The FDA stipulates in the Bioavailability and Bioequivalence Regulations that the most accurate, sensitive, and reproducible method for determining bioequivalence is to measure drug concentrations in blood in a single-dose study using human subjects. If it is not possible to accurately and reproducibly measure drug concentrations in blood, other approaches may be used, such as measuring an active metabolite or measuring drug in urine. For locally acting drug products with little systemic availability, bioequivalence may be evaluated by pharmacodynamic, clinical-endpoint, or highly specialized in vitro studies. Because of the challenges of the therapeutic equivalence criteria, there is not yet a mechanism for approving generic versions of many complex drug substances such as proteins, botanicals, and complex mixtures.

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Regulatory Considerations for Oral Extended Release Dosage Forms and in vitro (Dissolution)/in vivo (Bioavailability) Correlations

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INTRODUCTION

Optimizing drug therapy to patients is one of the important topics on the minds of all health care personnel. Drug developers, prescribers, and pharmacists would like to give the best drug to the patients, delivered in the most optimal way, to be taken the least number of times per day with maximized efficacy and minimal side effects. In this regard, modified-release dosage forms have found extensive use in today's pharmaceutical armamentarium. Due to technological developments in the pharmaceutical industry, advanced drug delivery systems are being developed to improve patient compliance (by needing to take the drug less frequently) and, in several cases, improved efficacy with reduced side effects. Modified-release dosage forms have thus become very popular in improving patient therapy. These dosage forms have sometimes also been developed to extend the patent life

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of the drug and drug product. The major goal in designing an extended release (ER) product should be that of optimizing therapeutic effects and safety of a drug, while at the same time improving patient convenience and compliance through extended dosage intervals. In this chapter, we will primarily focus on oral extended-release dosage forms, although the principles can be applied to nonoral extended-release products as well, e.g., transdermal systems. It is important to note that extended-release dosage forms are more complex than immediate-release dosage forms. Generally one dosage unit of extended-release product contains multiples of doses contained in an immediate-release dosage unit. In addition, the release of the drug from the extended-release product is intentionally modified. Therefore, it becomes extremely important to understand the release characteristics of these products as well as to evaluate how stable the release is under altered conditions in vivo, e.g., different pH, presence of food, etc. Because of these complexities involved in extended-release products, it is necessary to understand the regulatory considerations in evaluating these drug products.

In this chapter, we will first provide definitions and then discuss the regulatory considerations (in vivo and in vitro studies needed) for developing and maintaining oral extended- release products on the market. Finally, we will focus on in vitro/in vivo correlations to select meaningful dissolution methods that will enable the dissolution test to be a surrogate for bioequivalence. In this regard, we will provide several illustrations that will help understand the regulatory considerations as well as highlight some of the issues and pitfalls that arise in in vitro/in vivo correlations (IVIVC) development/validation.

DEFINITIONS

For ease of understanding, it is important to define the following terms before a substantial discussion of extended-release product development is started.

Controlled-Release Dosage Forms

A class of pharmaceuticals or other biologically active products from which a drug is released from the delivery system in a planned, predictable, and slower than normal or conventional manner (e.g., Ocuserts, Depot injectables such as Lupron depot) [1].

Modified-Release Dosage Forms

Dosage forms whose drug-release characteristics of time course and/or location are chosen to accomplish therapeutic or convenience objectives not offered by

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conventional dosage forms such as a solution or an immediate-release dosage form. Modified-release solid oral dosage forms include both delayed (e.g., enteric-coated products) and extended-release drug products [2].

Extended Release

Extended-release products are formulated to make the drug available over an extended period after ingestion. This allows a reduction in dosing frequency compared to a drug presented as a conventional dosage form (e.g., as a solution or an immediate-release dosage form) [2].

Delayed Release

Release of a drug at a time other than immediately following oral administration e.g., enteric coated products [2].

Compositionally Proportional

All active and inactive ingredients are in exactly the same proportion between different strengths (e.g., a tablet of 50-mg strength has all the inactive ingredients, exactly half that of a tablet of 100-mg strength, and twice that of a tablet of 25-mg strength).

Proportionally Similar

The phrase *proportionally similar* is defined in three ways [3]:

Definition 1 (compositionally proportional): All active and inactive ingredients are in exactly the same proportion between different strengths (e.g., a tablet of 50-mg strength has all the inactive ingredients, exactly half that of a tablet of 100-mg strength, and twice that of a tablet of 25-mg strength).

Definition 2: Active and inactive ingredients are not in exactly the same proportion between different strengths as stated above, but the ratios of inactive ingredients to total weight of the dosage form are within the limits defined by the SUPAC-IR and SUPAC-MR guidances up to and including Level II.

Definition 3: For high potency drug substances, where the amount of the active drug substance in the dosage form is relatively low, the total weight of the dosage form remains nearly the same for all strengths (within ±10% of the total weight of the strength on which a biostudy was performed), the same inactive ingredients are used for all strengths, and the change in any strength is obtained by altering the amount of the active ingredients and one or more of the inactive ingredients. The changes in the inactive ingredients

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are within the limits defined by the SUPAC-IR and SUP AC-MR guidances up to and including Level II.

In vitro/in vivo Correlations

A predictive mathematical model describing the relationship between an in vitro property of an oral dosage form (usually the rate or extent of drug dissolution or release) and a relevant in vivo response (e.g., plasma drug concentrations or amount of drug absorbed) [4].

FDA BIOAVAILABILITY STUDY REQUIREMENTS FOR CONTROLLED-RELEASE PRODUCTS—CODE OF FEDERAL REGULATIONS

The general pharmacokinetic/biopharmaceutic requirements for controlled-release formulations are set forth in 21 CFR 320.25(f) and are listed below (see the chapter on CFR):

21 CFR 320.25(f): Controlled-Release Formulations [5]

1. The purpose of an in vivo bioavailability study involving a drug product for which a controlled-release claim is made is to determine if all of the following conditions are met:

i. The drug product meets the controlled-release claims made for it.
ii. The bioavailability profile established for the drug product rules out the occurrence of any dose dumping.
iii. The drug product's steady-state performance is equivalent to a currently marketed noncontrolled-release or controlled-release drug product that contains the same active drug ingredient or therapeutic moiety and that is subject to an approved full new drug application.
iv. The drug product's formulation provides consistent pharmacokinetic performance between individual dosage units.

The types of studies needed to address these aspects are described in the next section.

2. The reference material(s) for such a bioavailability study shall be chosen to permit an appropriate scientific evaluation of the controlled-release claims made for the drug product. The reference material could be:

i. A solution or suspension of the active drug ingredient or therapeutic moiety.

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ii. A currently marketed noncontrolled-release drug product containing the same active drug ingredient or therapeutic moiety and administered according to the dosage recommendations in its labeling.
iii. A currently marketed controlled-release drug product subject to an approved full NDA containing the same active drug ingredient or therapeutic moiety and administered according to the dosage recommendations in its labeling.
iv. A reference material other than those discussed above that is appropriate for valid scientific reasons.

Clinical Pharmacology and Biopharmaceutics Studies

For extended-release dosage forms, the general studies needed are listed below [3, 6]. The first three studies listed are always necessary to address the CFR requirements.

1. Single-dose fasting relative bioavailability/bioequivalence study compared to a reference formulation
2. Steady-state relative bioavailability/bioequivalence study compared to a reference formulation
3. Food—effect study
4. Dose-proportionality study
5. Dosage strength bioequivalence study
6. Single-dose bioequivalence study (clinical vs. market formulations)
7. IVIVC
8. PK/PD evaluation

NEW DRUG APPLICATIONS VS. ABBREVIATED NEW DRUG APPLICATIONS

Some important considerations in deciding whether an ER dosage form should be filed as a new drug under a new drug application (NDA) or as a generic under an abbreviated new drug application (ANDA) are:

- Whether this drug is a new molecular entity
- Whether this ER product is the first extended-release product for that drug
- Whether there is any other similar ER product on the market
- Whether the sponsor intends to make claims of different efficacy or safety profile for this ER product

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In all the above cases, generally the ER product is submitted as an NDA. In situations where there is already an immediate-release form of the drug that is marketed, a 505(b)(2) NDA application could be submitted to the FDA for approval. These regulations for a 505(b)(2) NDA are covered under 21 CFR 314.54. Any person seeking approval of a drug product that represents a modification of a listed drug (e.g., a new indication or new dosage form) and for which investigations, other than bioavailability or bioequivalence studies, are essential to the approval of the changes may submit a 505(b)(2) application (except for cases where the only difference between the reference-listed drug and the test drug is that the extent of absorption is less than the reference or if the rate of absorption is unintentionally less than the reference). This application needs to contain only that information needed to support the modification(s) of the listed drug. If, however, the drug is already available as an ER product and the new sponsor is developing another ER product with no intention of being different from the currently marketed ER product, this will have to be submitted as an ANDA where one could rely solely on bioequivalence studies.

GENERAL APPROACHES FOR EVALUATING EXTENDED-RELEASE PRODUCTS

Are clinical trials always necessary for the approval of an ER product or can we rely on pharmacokinetic data alone? This is a fundamental question in evaluating ER products. A rational answer to this question is based on evaluation of the pharmacokinetic properties and plasma concentration/effect relationship of the drug. If there is a well-defined predictive relationship between the plasma concentrations of the drug and the clinical response (PK/PD for both safety and efficacy), it may be possible to rely on plasma concentration data alone as a basis for approval of the extended-release product. In the following situations, it is expected that clinical safety and efficacy data be submitted for approval of the ER product NDA:

- When the ER product involves a drug which has not previously been approved (in any dosage form), since there is no approved reference product to which a bioequivalence claim could be made
- When the rate of input has an effect on the drug's efficacy and safety profile
- When a claim of therapeutic advantage is intended for the ER product
- When there are safety concerns with regard to irreversible toxicity
- When there are uncertainties concerning the PK/PD relationships of the drug

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- Where there is evidence of functional (pharmacodynamic) tolerance
- Where peak to trough differences of the immediate-release dosage form are very large and the effect of input rate is unknown.

In vivo Studies Generally Necessary for Approval of ER NDAs

In cases where a new drug does not have adequate safety and efficacy established for either IR or ER dosage forms, safety and efficacy trials are required for an ER product. An example of such a case is where an ER product is being developed as the first dosage form of a new drug without prior approval or study of an IR product. As noted below, PK and PK/PD approaches may alleviate the need to conduct all of the usual safety and efficacy studies (i.e., a complete clinical trial program with two clinical efficacy and safety trials) for an ER product when an IR product is already approved.

The general approaches for studying and evaluating ER products are described below:

Demonstration of Safety and Efficacy Primarily based on Clinical Trials

- In general, for drugs where the concentration-response relationships are not established or are unknown, applications for an ER product where an IR product already exists will require the demonstration of the safety and efficacy of the product in the target patient population. In these cases, the PK and biopharmaceutics studies conducted to address the CFR requirements (described in the previous section) while necessary are mostly supportive and are usually for descriptive and labeling purposes. These studies may also help in the initial-dose selection.

When a new molecular entity is developed as an ER formulation, additional studies to characterize its clinical pharmacology and ADME characteristics will be necessary.

Demonstration of Safety and Efficacy based on PK, PK/PD, and Supportive Clinical Trials

The FDA "Guidance for Industry—Providing Clinical Evidence of Effectiveness for Human Drug and Biologic Products" [7] indicates that in certain cases, the clinical efficacy of modified-release dosage forms or different dosage forms can be extrapolated from existing studies, without the need for additional well-controlled clinical trials. This may be possible

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because other types of data such as PK studies (BA/BE studies) and/or PK/PD studies allow the application of known effectiveness to the new dosage form.

- "Where blood levels and exposure are not very different, it may be possible to conclude that a new form is effective on the basis of PK data alone."
- "Where blood levels are quite different, if there is a well-understood relationship between blood concentration and response, including an understanding of the time course of that relationship, it may be possible to conclude that the new dosage form is effective on the basis of pharmacokinetic data without an additional clinical efficacy trial."

The types of studies generally necessary in such cases will depend on the existence and nature of exposure-response relationships, and whether a therapeutic window has been established. The following cases provide some general ideas as to what studies and criteria may need to be met.

There is no prior knowledge of a concentration or exposure—response relationship or of a therapeutic window; approval is based solely on plasma profile comparisons and BE comparisons of PK parameters. Generally clinical trial(s) are necessary for approval in the case where there is no exposure-response relationship or a therapeutic window. An approach based solely on pharmacokinetic data *with* minimum or no information on PK/PD relationships is not generally encouraged. If it is agreed that the approval will be entirely based on PK data (e.g., based on prior knowledge of drug or its extensive use, or another appropriate reason agreed with FDA), bioequivalence between the IR and ER product is required in terms of C_{max}, C_{min}, and AUC at steady state. The overall plasma profile over the ER product's dosage interval must also be quite similar to the IR product's profile over the same time period. Differences in shapes of the plasma profiles may affect the efficacy and safety profiles of the drug. In such cases, the differences in shapes may outweigh findings of BE based on C_{max}, C_{min}, and AUC. If deviations in the steady-state PK profiles are seen between the ER and IR product regimens, additional PK/PD information or clinical studies may be required.

In certain cases, it may also be important to assess differences in steady-state t_{max} between the ER and IR products for approval purposes. Additional BA studies as previously outlined would also be required.

There is no quantitative concentration or exposure-response relationship but a well-defined therapeutic window in terms of safety and efficacy exists.

1. Case where the rate of input is known not to influence the safety and efficacy profile: When a therapeutic window that is well

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accepted exists and rate of input does not affect the safety/efficacy profile of the drug, the following criteria may be appropriate for comparing extended-release products to its reference (Note: there is no specific FDA Guidance that addresses this):

- For AUC_{ss}, the 90% confidence interval for the log-transformed ratio should be between 80–125
- The $C_{max\ ss}$ should be equal to or below the upper limit of the defined therapeutic window and the absolute $C_{min\ ss}$ should be equal to or above the lower limit of the defined therapeutic window.

Additional BA studies as previously outlined would also be necessary.

2. Case where it is unknown whether the rate of drug input influences the safety or efficacy profiles of the drug:

   Criteria can be the same as subcase 1, but in addition, studies investigating the impact of the rate of input on the pharmacodynamics of the drug in terms of safety and efficacy should be conducted and shown to have no rate effect.

   Additional BA studies as previously outlined would also be necessary.

There is a well-defined quantitative exposure-response relationship shown using different input rates or developed using the ER product.

1. If a concentration, or exposure-response relationship is established with the intended clinical endpoint and the safety profile of the drug is well understood, clinical safety and efficacy studies on the ER product may not generally be necessary. Acceptance criteria can be based on predictions of the clinical response from the steady-state plasma concentration time profile. Additional BA studies as previously outlined would also be required.
2. If a concentration, or exposure-response relationship is established with a validated surrogate measure, which is accepted as a validated marker for clinical efficacy, and the safety profile of the drug is well understood, clinical safety and efficacy studies may not generally be necessary. Acceptance criteria can be based on predictions of the clinical response from the plasma concentration profile. Additional BA studies as previously outlined would also be required.

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GENERAL CONSIDERATIONS IN EVALUATING PK/PD RELATIONSHIPS. In assessing PK/PD relationships, it is important to establish concentration-effect relationships and to determine the significance of differences in the shape of the steady-state concentration vs. time profile for an ER product regimen as compared to the approved IR product regimen. In this regard, any differential effects based on the rate of absorption and/or the fluctuation within a profile as related to safety and/or efficacy should be determined. Issues of tolerance to therapeutic effects and toxic effects related to drug exposure, concentration, absorption rate, and fluctuation should also be examined as part of the PK/PD assessment. In certain cases minimizing fluctuation in a steady-state profile for an ER product may be desirable to reduce toxicity while maintaining efficacy as compared to the IR product regimen (e.g., theophylline products). In other cases, minimizing fluctuation in a steady-state profile for an ER product may reduce efficacy (e.g., nitroglycerin—due to tolerance) as compared to the IR product regimen's profile where higher fluctuation is observed. It is therefore important to know the profile shape vs. PD relationships.

Safety Assessment of ER Dosage Form

Studies to assess the safety of the ER dosage form are generally necessary. An example of dosage unit or dosage unit/drug safety problems could be bezoar formation from some ER formulations.

In vivo Studies Needed for Approval of ER ANDAs (Generics) [3]

- A single-dose nonreplicate design fasting study comparing the test and reference-listed drug product. Since single-dose studies are considered to be most sensitive in addressing the primary question of bioequivalence [8] i.e., release of the drug at the same rate and to the same extent, multiple-dose BE studies are no longer necessary. For extended-release products marketed in multiple strengths, a single-dose bioequivalence study under fasting conditions is required only on the highest strength if all the strengths are proportionally similar and all strengths are manufactured under the same conditions. Bioequivalence studies on the lower strengths may be waived based on in vitro dissolution profiles. If the strengths are not proportionally similar, a single-dose bioequivalence study is required for each strength. This requirement can, however, be waived in the presence of an acceptable in vitro/in vivo correlation [4].

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- A fed state nonreplicate design bioequivalence study comparing the highest strength of the test and reference product [3].

In vitro Studies Needed (Dissolution)

Dissolution testing should be conducted on the ER product batches that were used in the pivotal BA/BE studies. The dissolution method should be appropriately selected after evaluation of several dissolution media (different pH) and agitation speeds, and should have adequate discriminatory power to differentiate between optimal and suboptimal batches. The sponsors are encouraged to develop dissolution methods that correlate with in vivo performance. If bio waivers for lower strengths are requested, adequate dissolution data needs to be submitted. Details of dissolution testing for ER products [2–4] can be found in the FDA "Guidance for Industry—Extended Release Oral Dosage Forms: Development, Evaluation, and Applications of in vitro/in vivo Correlations."

POSTAPPROVAL CHANGES

Refer to SUPAC-MR guidance, IVIVC (next section), and biowaivers chapter for details. In general, when manufacturing changes are made to an approved extended-release product, e.g., changes in composition, manufacturing site, batch size, equipment, process, etc., the requirements are defined under the FDA guidance "Scale-up and post approval changes for modified release dosage forms" [2]. In cases when the SUPAC-MR Guidance recommends a biostudy to support the change, an adequate in vitro/in vivo correlation can be used as justification. These are clearly explained in the FDA guidance on IVIVC (Extended release oral dosage forms: Development, evaluation and applications of in vitro/in vivo correlations [4]).

IVIVC [IN VITRO (DISSOLUTION)/IN VIVO (BIOAVAILABILITY) CORRELATIONS] [4, 9, 12, 13]

Why are IVIVCs Important?

In vitro dissolution has been extensively used as a quality control tool for solid oral dosage forms. Many times, however, it is not known whether one can predict the in vivo performance of these products from in vitro dissolution data. In an effort to minimize unnecessary human testing, investigations of in vitro/in vivo correlations between in vitro dissolution and in vivo bioavailability are increasingly becoming an integral part of extended-release drug product development. This increased activity in developing IVIVCs indicates the value of IVIVCs to the pharmaceutical industry. Because of the

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scientific interest and the associated utility of IVIVC as a valuable tool, the U.S. Food and Drug Administration has published a Guidance in September 1997, titled Extended Release Oral Dosage Forms: Development, Evaluation and Applications of in vitro/in vivo Correlations. A predictive IVIVC enables in vitro dissolution to serve as a surrogate for in vivo bioequivalence testing. In vitro/in vivo correlations can be used in place of biostudies that may otherwise be required to demonstrate bioequivalence, when certain changes are made in formulation, equipment, manufacturing process, or the manufacturing site. In vitro/in vivo correlation development could lead to improved product quality (more meaningful dissolution specifications) and decreased regulatory burden (reduced biostudy requirements).

Principles

In order to successfully develop an IVIVC, dissolution or release from the formulation has to be the rate-limiting step in the sequence of steps leading to absorption of the drug into the systemic circulation. Further, to utilize this dissolution test as a surrogate for bioequivalence (where a relatively simple in vitro test is used in place of human testing), the IVIVC must be predictive of in vivo performance of the product.

Levels of Correlation

Four categories of IVIVCs (levels A, B, C, and multiple level C) have been described in the FDA guidance. In addition, a qualitative rank order correlation (level D) has also been described in the U.S. Pharmacopoeia.

Level A

A level "A" correlation represents a point-to- point relationship between in vitro dissolution and the in vivo input rate (e.g., the in vivo dissolution of the drug from the dosage form). Level A correlation refers to a predictive mathematical model for the relationship between the *entire* in vitro dissolution/release time course and the *entire* in vivo response time course, e.g., fraction absorbed vs. fraction dissolved (see Fig. 1). Generally these correlations are linear; however, nonlinear correlations are also acceptable. A level "A" correlation is considered to be the most informative and very useful from a regulatory point of view.

Level B

A level "B" correlation uses the principles of statistical moment analysis [10]. Level B correlation is a predictive mathematical model of the

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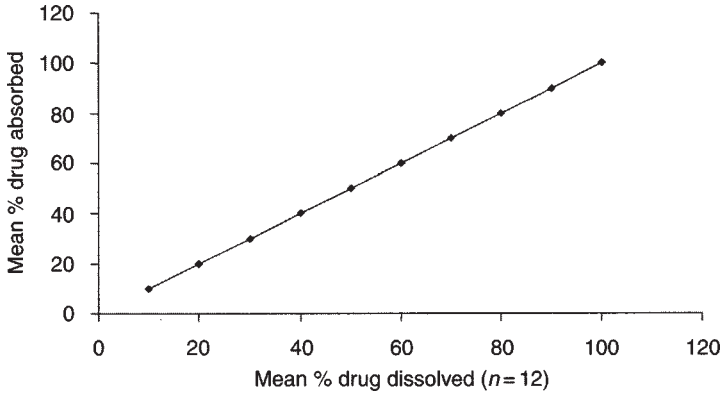

FIGURE 1 Level "A" correlation.

relationship between summary parameters (Fig. 2) that characterize the in vitro and in vivo time courses, e.g.,

a. mean in vitro dissolution time versus mean in vivo dissolution time
b. mean in vitro dissolution time versus mean residence time in vivo

Although this type of correlation uses all of the in vitro and in vivo data, it is not considered very useful since many different dissolution and plasma

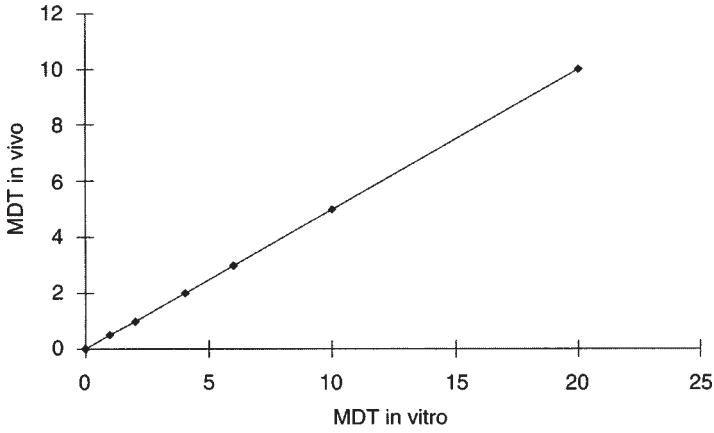

FIGURE 2 Level "B" correlation.

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concentration profiles and shapes can give the same mean summary parameters. Since it does not uniquely reflect the actual in vivo plasma level curve, this is not very useful from a regulatory point of view.

Level C

A level "C" correlation establishes a single-point relationship between a dissolution parameter (e.g., time for 50% dissolved or % dissolved in six hours) and a pharmacokinetic parameter (AUC and C_{max}) (Fig. 3).

A level "C" correlation does not reflect the complete shape of the plasma concentration time curve, therefore is not the most useful correlation from a regulatory point of view. However, this type of correlation can be useful in early formulation development.

Multiple Level C

A multiple level "C" correlation relates one or several pharmacokinetic parameters of interest to the amount of drug dissolved at several time points of the dissolution profile (e.g., C_{max} vs. % dissolved in two hours, six hours, and 12 hours)—see Fig. 4 below demonstrating a multiple level C correlation using formulations I to P [11]. This might be accomplished via linear regression. Multiple level "C" correlation can be as useful as level "A" IVIVC from a regulatory point of view. However, if one can develop a multiple level "C" correlation, it is likely that a level "A" correlation can be developed as well.

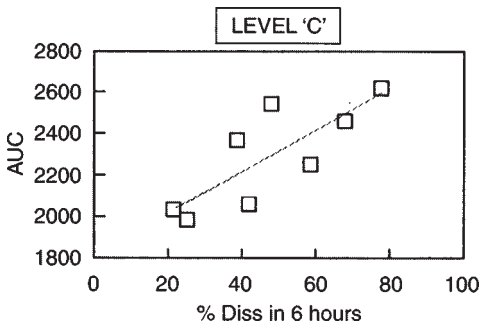

FIGURE 3 Level "C" correlation.

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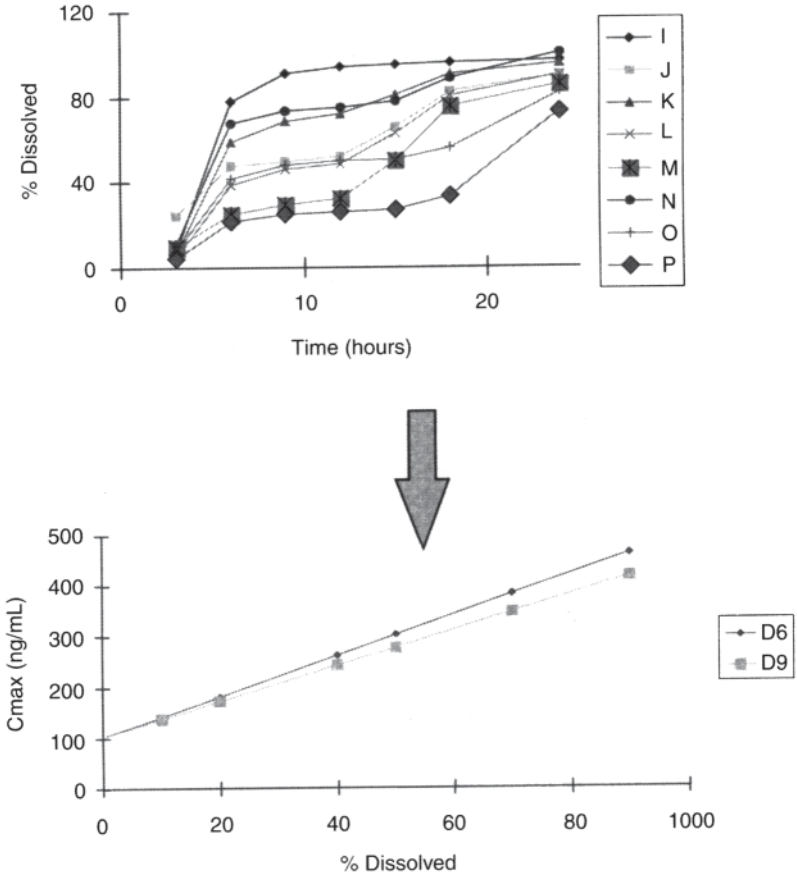

FIGURE 4 Multiple level "C" correlation.

When is an IVIVC Likely?

In vitro/in vivo correlations are generally seen when the dissolution or release of the drug from the dosage form is the rate-limiting step in the absorption and appearance of the drug in in vivo circulation.

FDA Guidance, "Extended Release Oral Dosage Forms: Development, Evaluation and Applications of in vitro/in vivo Correlations" [4]

This guidance has been developed (1) to reduce the regulatory burden by decreasing the number of biostudies needed to approve and maintain an

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extended-release product on the market and (2) to set clinically more meaningful dissolution specifications. It is anticipated that with a predictive IVIVC, the biostudies that are generally required for major manufacturing changes are replaced by a simple in vitro dissolution test.

General Principles/Considerations

The following general considerations apply in the development of an IVIVC:

- Human data are necessary for regulatory consideration of an IVIVC.
- Bioavailability studies for IVIVC development should be performed with enough subjects to characterize adequately the performance of the drug product under study. The number of subjects in some established IVIVCs has ranged from 6 to 36. Although crossover studies are preferred, parallel studies or cross-study analyses (with appropriate normalization with a common reference) may be acceptable. The reference product in developing an IVIVC may be an intravenous solution, an aqueous oral solution, or an immediate-release product.
- In vitro/in vivo correlations should usually be developed in the fasted state, unless the drug is not tolerated in fasted state and is indicated to be taken only in fed state due to tolerability concerns.
- Any in vitro dissolution method may be used to obtain the dissolution characteristics of the ER dosage form. The most common dissolution apparatus is USP apparatus I (basket) or II (paddle), used at compendially recognized rotation speeds (e.g., 100 rpm for the basket and 50–75 rpm for the paddle). An aqueous medium, either water or a buffered solution preferably not exceeding pH 6.8, is recommended as the initial medium for development of an IVIVC. For poorly soluble drugs, addition of surfactant (e.g., sodium lauryl sulfate) may be appropriate. Nonaqueous and hydroalcoholic systems are generally discouraged. The dissolution profiles of at least 12 individual dosage units from each lot should be determined.
- Generally, IVIVC should be developed using two or more formulations with different release rates. When two or more drug product formulations with different release rates are developed, their in vitro dissolution profiles should be generated using an appropriate dissolution methodology. The dissolution method used should be the same for all the formulations. The IVIVC relationship should be demonstrated consistently with

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two or more formulations with **different release rates** to result in corresponding differences in absorption profiles. [9, 12]. When in vitro dissolution is independent of the dissolution test conditions (e.g., medium, agitation, pH), development of IVIVC using one release rate formulation may be sufficient.

- An important factor is the range of release rates to study. The in vitro and in vivo profiles of the formulations used to develop IVIVC should be adequately different.
- Dissolution testing can be carried out during the formulation screening stage using several methods. Once a discriminating system is developed, dissolution conditions should be the same for all formulations tested in the biostudy for development of the correlation and should be fixed before further steps towards correlation evaluation are undertaken.
- It is important to note that the relationship between in vitro dissolution and in vivo dissolution, or absorption, should be the same for all the formulations studied. If one or more of the formulations (highest or lowest release rate formulations) does not show the same relationship between in vitro dissolution and in vivo performance compared with the other formulations, the correlation may still be used within the range of release rates encompassed by the remaining formulations.

IVIVC Development

The initial stage of establishing an IVIVC is an exploratory modeling process. One method to develop a level "A" correlation is to estimate the in vivo absorption or dissolution time course using an appropriate deconvolution technique for each formulation and subject (using Wagner-Nelson method, numerical deconvolution, etc.). The in vivo absorption profile is plotted against the in vitro dissolution profile to obtain a correlation (see Figs. 5 and 6).

A Level "A" correlation is usually estimated by a two-stage procedure: deconvolution followed by comparison of the fraction of drug absorbed to the fraction of drug dissolved [12]. Details of the deconvolution/convolution methodology can be found in several literature articles [14–17] and will not be discussed here. One alternative is based on a convolution procedure that models the relationship between in vitro dissolution and plasma concentration in a single step. Plasma concentrations predicted from the model and those observed are compared directly. For these methods, a reference treatment is desirable, but the lack of one does not preclude the ability to develop an IVIVC [16]. Whatever the method used to develop a Level "A" IVIVC, the IVIVC model should predict the entire in vivo time

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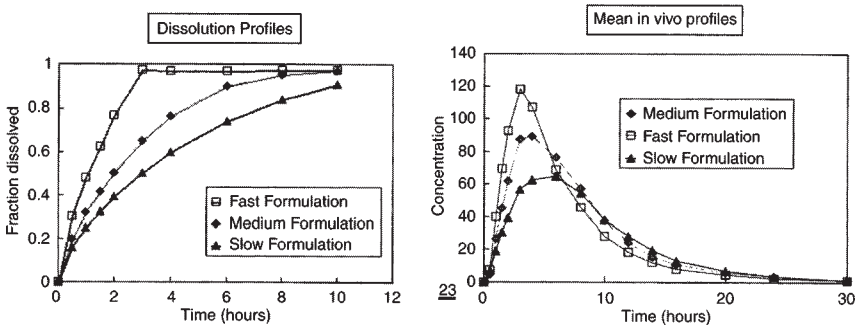

FIGURE 5 In vitro dissolution and in vivo profiles.

course from the in vitro data. Here the model refers to the relationship between in vitro dissolution of an ER dosage form and an in vivo response such as plasma drug concentration or amount of drug absorbed.

One could use alternative approaches than the ones mentioned to develop correlations. Also, if there is no one-to-one relationship, then dissolution conditions may be altered (prior to evaluation of predictability), or time-scaling approaches [18] may be used to develop the correlation. However, the time-scaling factor should be the same for all formulations tested. Different time scales for each of the formulations indicate absence of an IVIVC.

Evaluation of Predictability of IVIVC (IVIVC Validation)

An IVIVC should be evaluated to demonstrate that the predictability of the in vivo performance of a drug product, from the in vitro dissolution characteristics of the drug product formulations, is maintained over a range of in vitro release rates. A correlation should predict the in vivo performance accurately and consistently. When such an IVIVC has been established, in vitro dissolution can be used confidently as a surrogate for in vivo bioavailability/bioequivalence of ER drug products. Since the focus of IVIVC evaluation is on the predictive performance of the model, prediction error is evaluated and used as the criteria for IVIVC evaluation in the FDA Guidance (Figs. 7 and 8). Depending on the intended application of an IVIVC and the therapeutic index of the drug, evaluation of predictability internally and/or externally may be appropriate. Evaluation of internal predictability is based on the initial data used to develop the IVIVC. Evaluation of external predictability is based on additional data sets. External predictability evaluation is not necessary unless the drug is a

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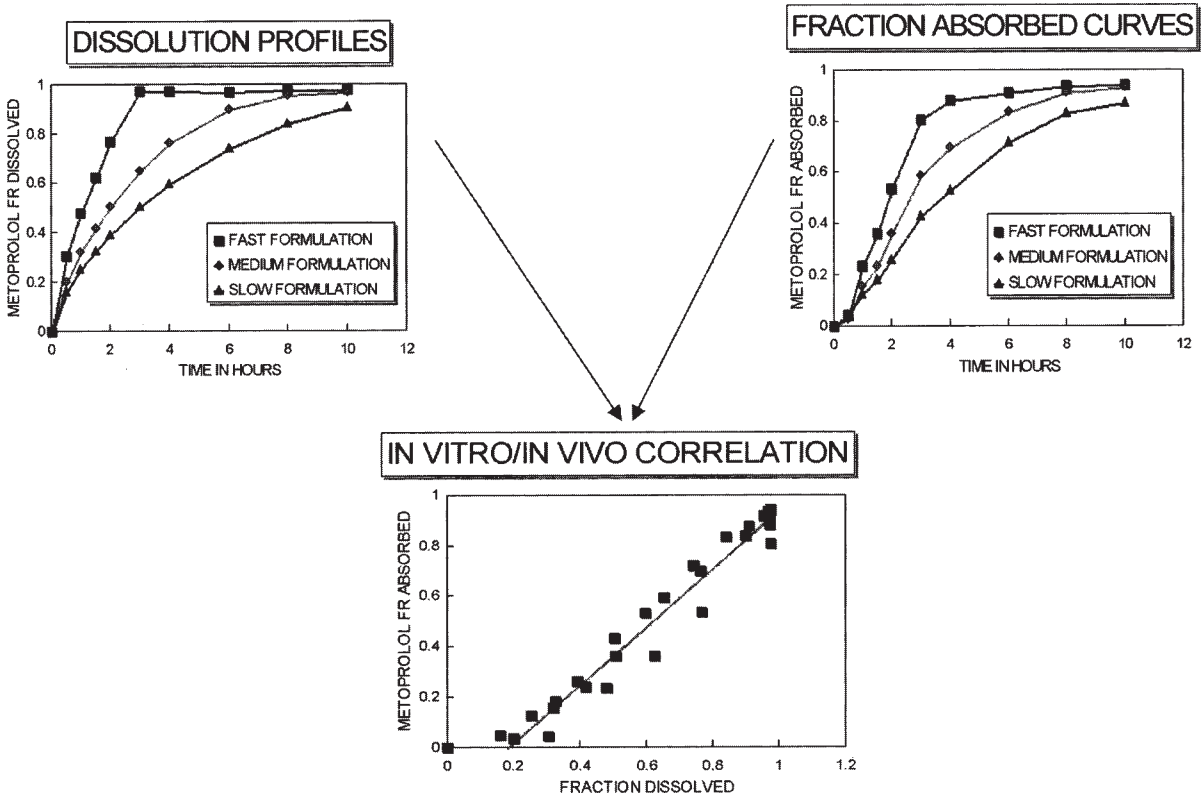

FIGURE 6 IVIVC development.

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narrow therapeutic index drug, or only two release rates were used to develop the IVIVC, or if the internal predictability criteria are not met (for criteria, see p. 438). However, since the IVIVC will potentially be used to predict the in vivo performance for future changes, it is of value to evaluate external predictability when additional data are available. An important concept is that the less data available for initial IVIVC development, the more additional data may be needed to define completely the IVIVC's predictability. Some combination of three or more formulations with different release rates is considered optimal.

Internal and External Predictability. Estimation of prediction error internally: Internal predictability should be evaluated for all IVIVCs (irrespective of the therapeutic index of the drug).

Estimation of prediction error externally. This is appropriate in some situations, particularly when only two formulations with different release rates are used to develop the IVIVC model, when calculation of prediction error internally is inconclusive, or when a narrow therapeutic index drug is studied.

The additional test data sets used for external prediction error calculation may have several differing characteristics compared to the data sets used in IVIVC development. Although formulations with different release rates provide the optimal test of an IVIVC's predictability, data from other types of formulations may be considered. In each case, bioavailability data should be available for the data set under consideration.

The following represent, in decreasing order of preference, formulations that may be used to estimate prediction error externally:

- A formulation with a different release rate than those used in IVIVC development.
- A formulation with the same or similar release rate, but involving some change in the manufacture of this batch (e.g., composition, process, equipment, manufacturing site).
- A formulation with the same or similar release rate obtained from another batch/lot with no changes in manufacturing.

Methods and Criteria for Evaluation of Predictability. The objective of IVIVC evaluation is to estimate the magnitude of the error in predicting the in vivo bioavailability results from in vitro dissolution data. Any appropriate approach related to this objective may be used for evaluation of predictability. One approach is to predict the in vivo plasma concentration-time profile from the in vitro dissolution data. This procedure is shown in Fig. 7 below, where the in vitro dissolution rate is converted to absorption rate using the IVIVC model and then convolved to predict the plasma

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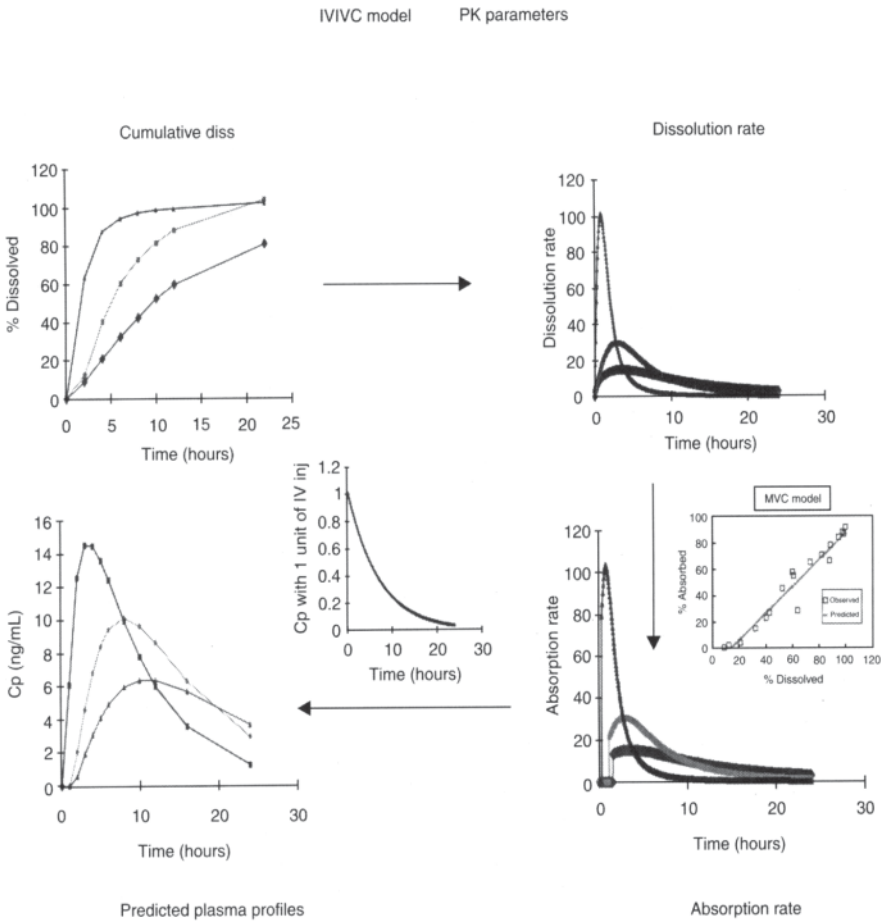

FIGURE 7 Prediction of in vivo profiles from in vitro dissolution data.

profile. The C_{max} and AUC from the predicted profiles should be compared to those from the observed profile to calculate % prediction errors on C_{max} and AUC (Fig. 8).

Absolute % prediction error on C_{max} and AUC:

$$\left[\frac{|\text{obs} - \text{pred}|}{\text{obs}}\right] * 100$$

Internal predictability: The recommended approach involves the use of the IVIVC model to predict each formulation's (formulations used in developing

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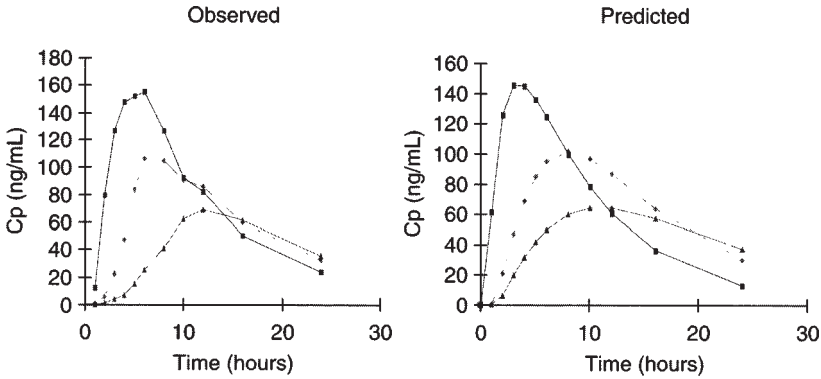

FIGURE 8 Comparsion of observed versus predicted profiles.

the IVIVC) plasma concentration profile (or C_{max} and/or AUC for a multiple level C IVIVC) from each respective formulation's dissolution data. Calculate the % prediction error on C_{max} and AUC.
Criteria

- Average absolute percent prediction error (% PE) of 10% or less for C_{max} and AUC establishes the predictability of the IVIVC. In addition, the % PE for each formulation should not exceed 15%.
- If these criteria are not met, that is, if the internal predictability of the IVIVC is inconclusive, evaluation of external predictability of the IVIVC should be performed as a final determination of the ability of the IVIVC to allow the use of in vitro dissolution as a surrogate for bioequivalence.

External predictability: This involves using the IVIVC to predict the in vivo performance of a formulation with known bioavailability that was not used in developing the IVIVC model.
Criteria

- The percent prediction error of 10% or less for C_{max} and AUC establishes the external predictability of an IVIVC.
- The percent prediction error between 10 and 20% indicates inconclusive predictability and the need for further study using additional data sets. Results of estimation of PE from all such data sets should be evaluated for consistency of predictability.
- The percent prediction error greater than 20% generally indicates inadequate predictability

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Caution During Evaluation of Predictability

In the evaluation of internal predictability, it is recommended that the PK parameter estimates used (e.g., for unit impulse response) in predicting the in vivo performance should be the average values or population estimates. Individual PK parameters should not be used to predict individual PK profiles which then are averaged to obtain the predicted average concentration—time profiles. This is due to the following three problems:

1. One does not have dissolution data on the dosage unit that the individual subject was administered. Therefore the input function is based on average parameters. Use of average in vitro parameters and individual in vivo parameters is not appropriate.
2. The percent prediction error calculated in this manner for internal predictability will always look better since the IVIVC was developed using the same individual values, and one is trying to predict the same data using the same individual estimates.
3. Further, since IVIVC will be used to obtain bio waivers when changes are made in future, based on in vitro dissolution data (and no in vivo data), one does not know what the individual parameters will be in each patient that is likely to use the drug. Therefore use of population estimates or mean PK parameters is recommended.

Applications of IVIVC

A predictive IVIVC can empower in vitro dissolution to act as a surrogate for in vivo bioavailability/bioequivalence. This can be used to grant biowaivers and to set meaningful dissolution specifications that take into account the clinical consequences.

Biowaivers. The Guidance outlines five categories of biowaivers. These are described in detail below.

1. Biowaivers without an IVIVC.
2. Biowaivers using an IVIVC: Nonnarrow therapeutic index drugs.
3. Biowaivers using an IVIVC: Narrow therapeutic index drugs.
4. Biowaivers when in vitro dissolution is independent of dissolution test conditions.
5. Situations for which an IVIVC is not recommended for biowaivers.

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Ideally, one would like to be able to predict the in vivo performance of the drug product from its in vitro dissolution. Therefore, with a predictive IVIVC, waivers for in vivo bioavailability studies may be granted for manufacturing site changes, equipment changes, manufacturing process changes, and formulation composition changes. The biowaivers section deals with changes ranging from situations such as minor changes, which are insignificant for product performance, to major changes for which an IVIVC is not sufficient to justify the change, for a regulatory decision. The IVIVC guidance in this area complements the SUPAC-MR guidance (Scale Up and Post Approval Changes—Modified Release Dosage Forms) [2]. An IVIVC can be used to support those drug product changes in SUPAC-MR that might have required a biostudy. However, there are situations such as those outlined under category 5, where an IVIVC cannot be used.

The mechanism of drug release from the drug product should remain the same when changes are made to a formulation for an IVIVC to be applicable. If the release mechanism changes (e.g., from a diffusion-controlled release to an osmotic release; beads to a matrix tablet), a previously developed IVIVC is not applicable.

The two criteria for granting a biowaiver for a new formulation, where an IVIVC has been established, are that the differences in predicted means of Cmax and AUC are no more than 20% from that of the reference product and, where applicable, the new formulation meets the application or compendial dissolution specifications (see Fig. 9).

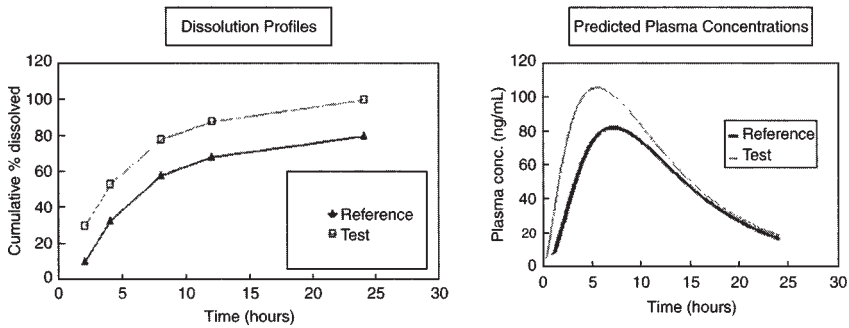

FIGURE 9 Prediction of in vivo profiles using IVIVC to grant biowaivers.

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Biowaivers with and without an IVIVC

Category 1: Biowaivers Without an IVIVC
This section relates to waivers for lower strengths (beaded capsules as well as tablets), changes made to lower strengths and certain preapproval changes—see biowaivers chapter and IVIVC Guidance for details.

Category 2: Biowaivers Using an IVIVC: Nonnarrow Therapeutic Index Drugs [4]

a. Two Formulations/Release Rates
A biowaiver is possible for an ER drug product using an IVIVC developed with two formulations/release rates for (1) Level 3 manufacturing site changes as defined in SUPAC-MR and (2) Level 3 nonrelease controlling excipient changes as defined in SUPAC-MR, with the exception of complete removal or replacement of excipients (see below).
b. Three Formulations/Release Rates
A biowaiver is possible for an ER drug product using an IVIVC developed with three formulations/release rates (or developed with two formulations/release rates with establishment of external predictability) for (1) Level 3 process changes as defined in SUPAC-MR; (2) complete removal of or replacement of nonrelease controlling excipients as defined in SUPAC-MR; and (3) Level 3 changes in the release controlling excipients as defined in SUPAC-MR.
c. Biowaivers for Lower Strengths
If an IVIVC is developed with the highest strength, waivers for changes made on the highest strength and any lower strengths may be granted if these strengths are compositionally proportional or qualitatively the same, the in vitro dissolution profiles of all the strengths are similar, and all strengths have the same release mechanism.
d. Biowaiver for New Strengths
This biowaiver is applicable generally to strengths lower than the highest strength (in some instances under an NDA (such as for compositionally proportional formulations), waiver for higher strengths may be possible if scientifically justified especially using an established IVIVC). For details on biowaiver and criteria for new strengths (in an NDA or an ANDA as a generic), see biowaivers chapter.
e. Changes in Release-Controlling Excipients
Changes in release-controlling excipients in the formulation should be within the quantitative range of release-controlling excipients

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(used in the different release rate formulations) of the established correlation.

f. Obtaining Category 2a, 2b, and 2c Biowaivers: The difference in predicted means of C_{max} and AUC should be no more than 20% from that of the reference product and, where appropriate, the new formulation should meet the application/compendial dissolution specifications.

Category 3: Biowaivers Using an IVIVC: Narrow Therapeutic Index Drugs [4]

If external predictability of an IVIVC is established, the following waivers (all waivers described under category 2 above including major site changes and nonrelease-controlling excipient changes) are possible if at least two formulations/release rates have been studied for the development of the IVIVC.

a. Manufacturing changes
A biowaiver is possible for an ER drug product using an IVIVC for (1) Level 3 process changes as defined in SUP AC-MR; (2) complete removal of or replacement of nonrelease-controlling excipients as defined in SUP AC-MR; and (3) Level 3 changes in the release-controlling excipients as defined in SUPAC-MR.

b. Biowaivers for Lower Strengths—see category 2c above for details

c. Approval of New Strengths—see category 2d above for details
Obtaining category 3c biowaivers: see requirements for obtaining 2d biowaivers

d. Changes in Release-Controlling Excipients—see category 2e above

e. Obtaining Category 3a and 3b Biowaivers: see requirements under category 2f above.

Category 4: Biowaivers When In Vitro Dissolution Is Independent of Dissolution Test Conditions [4]

Situations in which biowaivers are likely to be granted for both narrow and nonnarrow therapeutic index drugs:

a. Categories 2 and 3 biowaivers are likely to be granted with an IVIVC established with one formulation/release rate.

b. Obtaining Category 4 Biowaivers
   - Biowaivers may be granted if dissolution data are submitted in application/compendial medium and in three other media (e.g., water, 0.1 NHCl, USP buffer at pH 6.8) and the in vitro dissolution is shown to be independent of dissolution test

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conditions after the change is made in drug product manufacturing.

- The difference in predicted means of C_{max} and AUC should be no more than 20% from that of the reference product and, where appropriate, the new formulation should meet the application/compendial dissolution specifications. For new strengths, see 2d above.

Category 5: Situations for which an IVIVC Is Not Recommended [4]

a. Approval of a new formulation of an approved ER drug product when the new formulation has a different release mechanism.
b. Approval of a dosage strength higher or lower than the doses that have been shown to be safe and effective in clinical trials.
c. Approval of another sponsor's ER product even with the same release-controlling mechanism.
d. Approval of a formulation change involving a nonrelease-controlling excipient in the drug product that may significantly affect drug absorption.

Setting Dissolution Specifications [4]. Once an IVIVC is developed, this should be used to set dissolution specifications for the product. An IVIVC provides in vivo relevance to in vitro dissolution specifications, beyond batch-to-batch quality control. In this approach, the in vitro dissolution test becomes a meaningful predictor of in vivo performance of the formulation, and dissolution specifications may be used to minimize the possibility of releasing lots that would be different in in vivo performance.

1. Setting Dissolution Specifications Without an IVIVC

- The recommended range for dissolution specifications at any time point is ±10% of the label claim deviation from the mean dissolution profile obtained from the clinical/bioavailability batches. In certain cases, reasonable deviations from the ±10% range can be accepted provided that the range at any time point does not exceed 25%. Specifications greater than 25% may be acceptable based on evidence that lots (side batches) with mean dissolution profiles that are allowed by the upper and lower limits of the specifications are bioequivalent.
- A minimum of three time points are recommended to set the specifications. These time points should cover the early, middle, and late stages of the dissolution profile. The last time point

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should be the time point where at least 80% of drug has dissolved, or the time when the plateau of the dissolution profile has been reached.

- Specifications should be established based on average dissolution data (n= 12) for each lot under study, equivalent to USP Stage 2 testing. Specifications that allow all lots to pass at Stage 1 of testing may result in lots with less than optimal in vivo performance passing these specifications at USP Stage 2 or Stage 3. USP acceptance criteria for dissolution testing are recommended unless alternate acceptance criteria are specified in the ANDA/NDA.

2. Setting Dissolution Specifications Where an IVIVC Has Been Established

If an IVIVC has been established, it should be used to set dissolution specifications. Optimally, specifications should be established such that all lots that have dissolution profiles within the upper and lower limits of the specifications are bioequivalent. Less optimally but still possible, lots exhibiting dissolution profiles at the upper and lower dissolution limits should be bioequivalent to the clinical/bioavailability lots or to an appropriate reference standard.

a. Level A Correlation Established

- Specifications should be established based on average data (n=12).
- A minimum of three time points that cover the early, middle, and late stages of the dissolution profile is recommended to establish the specifications. The last time point should be the time point where at least 80% of drug has dissolved or the time where the plateau of the dissolution profile has been reached.
- Predict the plasma concentration time profile using convolution techniques or other appropriate modeling techniques and determine whether the lots with the fastest and slowest release rates that are allowed by the dissolution specifications result in a maximal difference of 20% in the predicted C_{max} and AUC (see Fig. 10). An established IVIVC may allow setting wider dissolution specifications. This would be dependent on the predictions of the IVIVC (i.e., 20% differences in the predicted C_{max} and AUC). However, if based on the IVIVC, the dissolution specifications justified are less than the 20% range allowed with an IVIVC, a minimum range of 20% will be generally allowed unless there are clinical concerns.
- USP acceptance criteria for dissolution testing are recommended unless alternate acceptance criteria are specified in the ANDA/NDA.

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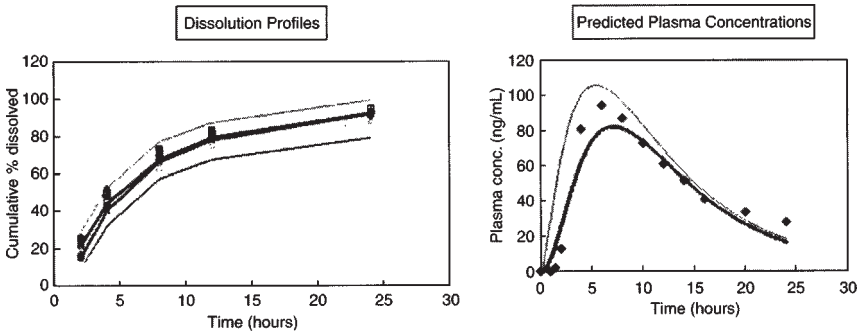

FIGURE 10 Setting dissolution specifications based on level "A" IVIVC.

b. Multiple Level C Correlation Established

If a multiple-point Level C IVIVC has been established, establish the specifications at each time point such that there is a maximal difference of 20% in the predicted C_{max} and AUC. Additionally, the last time point should be the time point where at least 80% of drug has dissolved.

c. Level C Correlation Based on Single Time Point Established

This one time point may be used to establish the specification such that there is not more than a 20% difference in the predicted AUC and C_{max}. At other time points, the maximum recommended range at any dissolution time point specification should be ±10% of label claim deviation from the mean dissolution profile obtained from the clinical/bioavailability lots. Reasonable deviations from ± 10% may be acceptable if the range at any time point does not exceed 25%.

3. Setting Specifications Based on Release Rate

If the release characteristics of the formulation can be described by a zero-order process for some period of time (e.g., 5%/hr from 4 to 12 hours), and the dissolution profile appears to fit a linear function for that period of time, a release-rate specification may be established to describe the dissolution characteristics of that formulation. Such a specification may provide for a better control of the in vivo performance of the product. A release rate specification may be (i) an addition to the specifications established on the cumulative amount dissolved at the selected time points, or (ii) may be the only specification along with a cumulative dissolution specification for time when at least 80% of drug has dissolved.

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Regulatory Impact of IVIVCs

IVIVC can impart in vivo meaning to the in vitro dissolution test and can be useful as surrogate for bioequivalence. IVIVCs can thus decrease regulatory burden by decreasing the number of biostudies required in support of a drug product. As an additional benefit to the sponsors, IVIVC can support wider in vitro dissolution specifications, where justified. FDA strongly encourages the development and evaluation of IVIVCs during ER product development. Generally IVIVC development adds value to the overall drug development process by providing an understanding of the relevance of the in vitro dissolution data leading to better utilization of the in vitro dissolution test. Usually this IVIVC development can be done without conducting new studies. One can use the early development studies where multiple release-rate formulations are generally incorporated in the bioavailability studies. IVIVCs can thus be useful in decreasing the regulatory burden with no undue penalty to the companies that develop these correlations.

EMEA GUIDANCE THAT DEALS WITH IVIVC [19]

The EMEA Guidance on Quality of MR products and transdermal products covers some of the considerations in development and evaluation of IVIVC and some applications of IVIVC. Similar to U.S. FDA, sponsors are asked to consider development of an IVIVC. If an IVIVC is established, the dissolution test, after proper validation, can be used as a "qualifying control method with in vivo relevance" rather than just a quality control test.

- Levels of correlations are defined in a similar manner to the FDA Guidance.
- Development of IVIVC: Development considerations of levels A, B, and C IVIVC are briefly discussed in this guidance. For a level A IVIVC, generally one formulation tested at different dissolution conditions should be compared to aqueous solution. This seems to be different (although not explicit) from the FDA Guidance where there is a need to study multiple release-rate formulations.
- Evaluation of predictability: Methods and criteria for predictability are the same as in the FDA Guidance; however, there is no explicit discussion of situations with condition-independent dissolution or narrow therapeutic index drugs.
- Applications—Biowaivers: While the FDA Guidance provides detailed situations for biowaivers, the EMEA Guidance provides a summary to state that when a Level A IVIVC has been established and the release specification is not changed, type II

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variations (e.g., major changes in nonrelease-controlling excipients, insignificant changes in release-controlling excipients or major changes in method of manufacturing) may be accepted on the basis of in vitro data, the therapeutic index of the drug substance and predictability of the IVIVC. In general, BA/BE data are needed for products with an established level B or C correlation or no IVIVC, unless justified.

- Applications—Dissolution specifications: If IVIVC is established, it is used to set specifications. However, there are some differences from the FDA Guidance.

  (A) Level A: The specification is based on a 1:1 correlation between the dissolution profile in vivo and in vitro (FDA Guidance is not restricted to a 1:1 correlation).
  (B) Level B correlation can also be used to set specifications, although methodology details are not provided (Level B correlations are not useful for waivers or setting dissolution specifications according to the FDA Guidance).
  (C) For any level of correlation, i.e., levels A, B, C, or multiple level C, specifications should be set such that the maximal difference in predicted AUC is 20% and, predicted C_{max} only if relevant (FDA Guidance requires both AUC and C_{max}).

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In vivo Bioavailability/Bioequivalence Waivers

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INTRODUCTION

Bioavailability (BA) is defined in 21 CFR 320.1 as "the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream [1], bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action." Bioequivalence (BE) is defined in 21 CFR 320.1 as "the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study." As noted in the statutory definitions, both BE and product quality BA focus on the release of a drug substance from a drug product and subsequent absorption into the systemic circulation [1]. Over the last 30

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years, dissolution testing has not only been recognized as a valuable quality control test but has also proved itself as a useful indicator of differences in bioavailability. This is due to the fact that drug absorption after oral administration depends on the release of the drug substance from the drug product, the dissolution or solubilization of the drug under physiological conditions and the permeability across the gastrointestinal tract. Whenever, a significant difference in bioavailability has been found among supposedly identical articles, the dissolution test most of the times has been able to discriminate among these articles. In fact, dissolution is so sensitive to formulation factors that bioequivalent formulations sometimes show differences in dissolution profiles. According to the regulations stated in CFR 320.24, bioavailability and bioequivalence could be assessed by several in vitro or in vivo methods depending on the purpose of the study, the availability of analytical methods, and the nature of the drug product. Specifically CFR 320.24 states that either an in vitro test that has been correlated with and is predictive of human bioavailability data or a currently available in vitro test acceptable to FDA that ensures that human in vivo bioavailability is acceptable [2]. This chapter starts with definitions followed by the relevant regulations governing in vivo bioavailability/bioequivalence waivers with a discussion on the various types of waivers based on comparability of dissolution profiles for both immediate-release (IR) dosage forms and modified-release (MR) dosage forms. Moreover, the types of scale up and postapproval changes that can be approved based on comparability of dissolution profiles are summarized for both IR and MR products. A brief description on how to compare dissolution profiles is given. The role of in vitro-in vivo correlations (IVIVC) for MR products as well as the biopharmaceutics classification system (BCS) for IR products in alleviating the regulatory burden is elucidated. Finally, an overview of the Japanese, European, and Canadian guidelines for instances where an in vivo BA/BE waiver can be granted based on comparability of dissolution profiles is provided.

DEFINITIONS

Proportionally Similar.
Definition 1: All active and inactive ingredients are in exactly the same proportion between different strengths (e.g., a tablet of 50-mg strength has all the inactive ingredients, exactly half that of a tablet of 100-mg strength, and twice that of a tablet of 25-mg strength).

Definition 2: Active and inactive ingredients are not in exactly the same proportion between different strengths as stated above, but the ratios of

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inactive ingredients to total weight of the dosage form are within the limits defined by the SUPAC-IR and SUP AC-MR guidances up to and including Level II.

Definition 3: For high potency drug substances, where the amount of the active drug substance in the dosage form is relatively low, the total weight of the dosage form remains nearly the same for all strengths (within ±10% of the total weight of the strength on which a biostudy was performed), the same inactive ingredients are used for all strengths, and the change in any strength is obtained by altering the amount of the active ingredients and one or more of the inactive ingredients. The changes in the inactive ingredients are within the limits defined by the SUPAC-IR and SUPAC-MR guidances up to and including Level II [3].

Delayed Release: As defined in the U.S. Pharmacopeia (USP), delayed-release drug products are dosage forms that release the drugs at a time later than immediately after administration (i.e., these drug products exhibit a lag time in quantifiable plasma concentrations) [4].

Extended-Release: These are dosage forms that allow a reduction in dosing frequency as compared to when the drug is present in an immediate-release dosage form. These drug products can also be developed to reduce fluctuations in plasma concentrations. Extended-release products can be capsules, tablets, granules, pellets, and suspensions [4].

Case A Dissolution: Amount dissolved equals 85% in 15 minutes in 900 mL of 0.1 N HC1 using USP apparatus 1 at 100 rpm or apparatus 2 at 50 rpm.

Case B Dissolution: Multipoint dissolution profile in the application/compendial medium at 15, 30, 45, 60, and 120 minutes or until either 90% of the drug from the drug product is dissolved or an asymptote is reached for the proposed and currently accepted formulation.

Case C Dissolution: Multipoint dissolution profiles performed in water, 0.1N HC1, and USP buffer at pH 4.5, 6.5, and 7.5 (five separate profiles) for the proposed and currently accepted formulations. Adequate sampling should be performed at 15, 30, 45, 60, and 120 minutes until either 90% of the drug from the drug product is dissolved or an asymptote is reached. A surfactant may be used with appropriate justification [5].

Pharmaceutical Equivalents: Drug products are considered pharmaceutical equivalents if they contain the same active ingredient(s), are of the same dosage form and route of administration, and are identical in strength and concentration [6].

Therapeutic Equivalents: Drug products are considered to be therapeutic equivalents only if they are pharmaceutical equivalents and if they can be expected to have the same clinical effect and safety profile when administered to patients under the conditions specified in the label.

Pharmaceutical Alternatives: Drug products are considered pharmaceutical

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alternatives if they contain the same therapeutic moiety or are different dosage forms or strengths [6].

CODE OF FEDERAL REGULATIONS

CFR 320.22 [7] gives FDA the authority under certain circumstances to waive the requirements for evidence for determining the in vivo bioavailability and bioequivalence. Specifically the CFR states:

a. Any person submitting a full or abbreviated new drug application, or a supplemental application proposing any of the changes set forth in Sec. 320.21(c), may request FDA to waive the requirement for the submission of evidence demonstrating the in vivo bioavailability or bioequivalence of the drug product that is the subject of the application. An applicant shall submit a request for waiver with the application. Except for certain situations, FDA shall waive the requirement for the submission of evidence of in vivo bioavailability or bioequivalence if the drug product meets any of the provisions of paragraphs (b), (c), (d), or (e) of this section.

b. For certain drug products, the in vivo bioavailability or bioequivalence of the drug product may be self-evident. FDA shall waive the requirement for the submission of evidence obtained in vivo demonstrating the bioavailability or bioequivalence of these drug products. A drug product's in vivo bioavailability or bioequivalence may be considered self-evident based on other data in the application if the product meets one of the following criteria:

    1. The drug product:

        i. Is a parenteral solution intended solely for administration by injection, or an ophthalmic or otic solution; and
        ii. Contains the same active and inactive ingredients in the same concentration as a drug product that is the subject of an approved full new drug application.

    2. The drug product:

        i. Is administered by inhalation as a gas, e.g., a medicinal or an inhalation anesthetic; and
        ii. Contains an active ingredient in the same dosage form as a drug product that is the subject of an approved full new drug application.

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3. The drug product:

    i. Is a solution for application to the skin, an oral solution, elixir, syrup, tincture, or similar other solubilized form.
    ii. Contains an active drug ingredient in the same concentration and dosage form as a drug product that is the subject of an approved full new drug application; and
    iii. Contains no inactive ingredient or other change in formulation from the drug product that is the subject of the approved full new drug application that may significantly affect absorption of the active drug ingredient or active moiety.

c. FDA shall waive the requirement for the submission of evidence demonstrating the in vivo bioavailability of a solid oral dosage form (other than an enteric coated or controlled-release dosage form) of a drug product determined to be effective for at least one indication in a Drug Efficacy Study Implementation notice or which is identical, related, or similar to such a drug product under Sec. 310.6 of this chapter unless FDA has evaluated the drug product under the criteria set forth in Sec. 320.32, included the drug product in the Approved Drug Products with Therapeutic Equivalence Evaluations List, and rated the drug product as having a known or potential bioequivalence problem. A drug product so rated reflects a determination by FDA that an in vivo bioequi valence study is required.

d. For certain drug products, bioavailability or bioequivalence may be demonstrated by evidence obtained in vitro in lieu of in vivo data. FDA shall waive the requirement for the submission of evidence obtained in vivo demonstrating the bioavailability of the drug product if the drug product meets one of the following criteria:

    1. The drug product is in the same dosage form, but in a different strength, and is proportionally similar in its active and inactive ingredients to another drug product for which the same manufacturer has obtained approval and the conditions in paragraphs (d)(2)(i) through (d)(2)(iii) of this section are met:

        i. The bioavailability of this other drug product has been demonstrated,
        ii. Both the drug products meet an appropriate in vitro test approved by FDA.
        iii. The applicant submits evidence showing that both drug

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products are proportionally similar in their active and inactive ingredients.

iv. This subparagraph does not apply to enteric coated or controlled-release dosage forms.

2. The drug product is, on the basis of scientific evidence submitted in the application, shown to meet an in vitro test that has been correlated with in vivo data.
3. The drug product is a reformulated product that is identical, except for a different color, flavor, or preservative that could not affect the bioavailability of the reformulated product, to another drug product for which the same manufacturer has obtained approval and the following conditions are met:

   i. The bioavailability of the other product has been demonstrated,
   ii. Both drug products meet an appropriate in vitro test approved by FDA.

e. FDA, for good cause, may waive a requirement for the submission of evidence of in vivo bioavailability if waiver is compatible with the protection of the public health. For full new drug applications, FDA may defer a requirement for the submission of evidence of in vivo bioavailability if deferral is compatible with the protection of the public health.
f. FDA, for good cause, may require evidence of in vivo bioavailability or bioequivalence for any drug product if the agency determines that any difference between the drug product and a listed drug may affect the bioavailability or bioequivalence of the drug product.

WAIVERS OF IN VIVO BIOAVAILABILITY/BIOEQUIVALENCE STUDIES WITHOUT IVIVC

Different Strengths

Immediate-release Drug Products

When the drug product is in the same dosage form, but in a different strength, and is proportionally similar in its active and inactive ingredients to that of a listed drug, an in vivo BE demonstration of one or more lower strengths can be waived based on dissolution tests and an in vivo study on the highest strength.

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For an NDA, biowaivers of a higher strength will be determined to be appropriate based on (1) clinical safety and/or efficacy studies including data on the dose and the desirability of the higher strength; (2) linear elimination kinetics over the therapeutic dose range; (3) the higher strength being proportionally similar to the lower strength; and (4) the same dissolution procedures being used for both strengths, and similar dissolution results obtained in the approved medium. If the dissolution medium has not been selected, then dissolution profiles in three media should be generated (0.1 N HC1, phosphate buffer pH 4.5 and 6.8). A dissolution profile should be generated for all strengths [3].

For an ANDA, conducting an in vivo study on a strength that is not the highest may be appropriate for reasons of safety, subject to approval by review staff. In addition, as with an NDA, the Agency will consider a waiver request for a recently approved higher strength when an in vivo BE study was performed on a lower strength of the same drug product submitted in an ANDA under the following circumstances:

- Linear elimination kinetics has been shown over the therapeutic dose range.
- The higher strength is proportionally similar to the lower strength.
- Comparative dissolution testing on the higher strength of the test and reference drug product is submitted and found acceptable.
- The sponsor initiated the BE study on the lower strength within five working days of the approval date of a higher strength of the reference-listed drug. A study is considered initiated when the first subject is dosed.

Sponsors of AND As wishing to submit a biowaiver request under these circumstances should first contact the Regulatory Support Branch, Office of Generic Drugs, for advice on the proper filing procedure.

Modified-release Drug Products

Beaded Capsules—Lower Strength. For extended-release beaded capsules, where the strength differs only in the number of beads containing the active moiety, a single-dose, fasting BE study can be carried out only on the highest strength, with a request for a waiver of in vivo studies for lower strengths based on dissolution profiles. A dissolution profile should be generated for each strength using the recommended dissolution method. The f_2 test should be used to compare profiles from the different strengths of the product. An f_2 value of ≥ 50 can be used to confirm that further in vivo studies are not needed.

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Tablets—Lower Strength. For extended-release tablets, when the drug product is in the same dosage form but in a different strength, is proportionally similar in its active and inactive ingredients, and has the same drug-release mechanism, an in vivo BE determination of one or more lower strengths can be waived based on dissolution profile comparisons, with an in vivo study only on the highest strength. The drug products should exhibit similar dissolution profiles between the highest strength and the lower strengths based on the f_2 test in at least three dissolution media (e.g., pH 1.2, 4.5, and 6.8). The dissolution profile should be generated on the test and reference products of all strengths [3].

Transdermal Patches

In vivo bioavailability/bioequivalence demonstration for lower strengths transdermal patches can be waived based on comparability of dissolution profiles in three media (0.1 N HC1, phosphate buffer pH 4.5 and 6.8) and the presence of an acceptable in vivo study on the highest strengths, provided that the lower strengths patches are compositionally proportional in all their components and are manufactured under the same manufacturing conditions at the same manufacturing site using the same equipment as in the case of highest strengths.

Clinical vs. Market Formulation

During the course of drug development, sponsors sometimes have to blind the formulations that they use in the clinical trials. In certain situations, the only difference between the market and clinical trial formulation is that the tablet mix or the tablet itself is put into a capsule. This is done mainly for blinding purposes. It is thus possible to get a waiver for the bioequivalence study that links the market and clinical trial formulation, provided that no other excipients are added to the capsule that are known to affect the release of the active drug from the capsule. The waiver of this in vivo bioequivalence study is granted based on the comparability of the dissolution profile in three media: 0.1 N HC1 and phosphate buffer pH 4.5 and 6.8.

Scale Up and Postapproval Changes

It is possible that postapproval and sometimes preapproval, a sponsor might make certain formulation changes in components and composition, scale up change, manufacturing site change, and manufacturing process or equipment change. Depending on the possible impact of the manufacturing change on the release of the active ingredient and its bioavailability from that formulation, certain manufacturing changes can be approved solely based

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on comparability of the dissolution profiles between the changed and unchanged formulation. Both guidances on Scale Up and Postapproval Changes for immediate-release formulations and for modified-release formulations define three levels of change.

According to these guidances, a level 1 change is a change that is unlikely to have any detectable impact on formulation quality and performance [5].

A level 2 change is defined as a change that could have a significant impact on formulation quality and performance. The amount of information required for the approval of such changes depends on the therapeutic window of the drug, its solubility, and permeability.

Level 3 changes are defined as changes that are likely to have a significant impact on formulation quality and performance.

In general, level 1 and 2 changes can be approved based on comparability of dissolution profiles while level 3 changes usually necessitate an in vivo bioequivalence study.

Tables 1 and 2 summarize the type of change that can be approved just based on in vitro dissolution data for IR and MR formulations, respectively [8].

TABLE 1 Summary of the in vitro Dissolution Data Requirements for the Manufacturing Changes for Immediate-Release Formulations for which an in vivo Bioavailability Waiver can be Obtained

Application/ compendial requirements	Case A	Case B	Case C
Level 1 component and composition	Level 2 component and composition for high permeability and high solubility drugs	Level 2 component and composition for low permeability and high solubility drugs	Level 2 component and composition for high permeability and low solubility drugs
Level 1 and 2 site change		Level 3 site change	Level 2 equipment change
Level 1 change in batch size		Level 2 change in batch size	
Level 1 equipment change		Level 2 and 3 process change	
Level 1 process change			

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TABLE 2 Summary of the In vitro Dissolution Data Requirements for the Manufacturing Changes for Modified-Release Formulations for which an in vivo Bioavailability Waiver can be Obtained

Application/compendial requirements	Application/compendial requirements + 0.1 N HCl for 2 hours followed by testing in USP buffer pH 4.5–7.5 under standard conditions + 2 additional agitation speeds using the compendial test apparatus	Application/compendial requirements + 3 additional media such as water, 0.1 N HCl, and phosphate buffer pH 4.5–7.5
Level 1 change in nonrelease-controlling excipients	Level 2 change in nonrelease-controlling excipients for delayed release	Level 2 change in nonrelease-controlling excipients for extended release
Level 1 change in release-controlling excipients	Level 2 change in release-controlling excipients for delayed release non narrow therapeutic drug	Level 2 change in release-controlling excipients for extended release non narrow therapeutic drug
Level 1 change in batch size	Level 2 site change for delayed release	Level 2 site change for extended release
Level 1 site change	Level 2 change in batch size for delayed release	Level 2 change in batch size for extended release
Level 1 equipment change	Level 2 manufacturing process change for delayed release	Level 2 manufacturing process change for extended release
Level 1 manufacturing process change	Level 2 change in equipment for delayed release	Level 2 change in equipment for extended release

DISSOLUTION PROFILE COMPARISONS

Dissolution profiles may be considered similar by virtue of (1) overall profile similarity and (2) similarity at every dissolution sample time point. The dissolution profile comparison may be carried out using model-independent or model-dependent methods.

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Model-independent Approach Using a Similarity Factor

A simple model-independent approach uses a difference factor *(f_1)* and a similarity factor *(f_2)* to compare dissolution profiles [5]. The difference factor *(f_1)* calculates the percent (%) difference between the two curves at each time point and is a measurement of the relative error between the two curves:

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right\} 100,$$

where n is the number of time points, R_t is the dissolution value of the reference (prechange) batch at time t, and T_t is the dissolution value of the test (postchange) batch at time t.

The similarity factor *(f_2)* is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the two curves.

$$f_2 = 50 \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} 100 \right\}$$

A specific procedure to determine difference and similarity factors is as follows:

1. Determine the dissolution profile of two products (12 units each) of the test (postchange) and reference (prechange) products.
2. Using the mean dissolution values from both the curves at each time interval, calculate the difference factor *(f_1)* and similarity factor *(f_2)* using the above equations.
3. For curves to be considered similar, f_1 values should be close to 0, and f_2 values should be close to 100. Generally, f_1 values up to 15 (0–15) and f_2 values greater than 50 (50–100) ensure sameness or equivalence of the two curves and, thus, of the performance of the test (postchange) and reference (prechange) products.

This model-independent method is most suitable for dissolution profile comparison when three to four or more dissolution time points are available. As further suggestions for the general approach, the following recommendations should also be considered: The dissolution measurements of the test and reference batches should be made under exactly the same conditions. The dissolution time points for both the profiles should be the same (e.g., 15, 30, 45, and 60 minutes). The reference batch used should be the most recently manufactured prechange product. Only one measurement

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should be considered after 85% dissolution of both the products. To allow use of mean data, the percent coefficient of variation at the earlier time points (e.g., 15 minutes) should not be more than 20%, and at other time points should not be more than 10%. The mean dissolution values for *R* can be derived either from (1) last prechange (reference) batch or (2) last two or more consecutively manufactured prechange batches.

Model-Independent Multivariate Confidence Region Procedure

In instances where within batch variation is more than 15% CV, a multivariate model-independent procedure is more suitable for dissolution profile comparison. The following steps are suggested:

1. Determine the similarity limits in terms of multivariate statistical distance (MSD) based on interbatch differences in dissolution from reference (standard approved) batches.
2. Estimate the MSD between the test and reference mean dissolutions.
3. Estimate 90% confidence interval of true MSD between test and reference batches.
4. Compare the upper limit of the confidence interval with the similarity limit.

The test batch is considered similar to the reference batch if the upper limit of the confidence interval is less than or equal to the similarity limit.

Model-Dependent Approaches

Several mathematical models have been described in the literature to fit dissolution profiles. To allow application of these models to comparison of dissolution profiles, the following procedures are suggested:

1. Select the most appropriate model for the dissolution profiles from the standard, prechange, approved batches. A model with no more than three parameters (such as linear, quadratic, logistic, probit, and Weibull models) is recommended.
2. Using data for the profile generated for each unit, fit the data to the most appropriate model.
3. A similarity region is set based on variation of parameters of the fitted model for test units (e.g., capsules or tablets) from the standard approved batches.
4. Calculate the MSD in model parameters between test and reference batches.

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5. Estimate the 90% confidence region of the true difference between the two batches.
6. Compare the limits of the confidence region with the similarity region. If the confidence region is within the limits of the similarity region, the test batch is considered to have a dissolution profile similar to that of the reference batch [7].

WAIVERS BASED ON IN VIVO-IN VITRO CORRELATION

For modified-release formulations, it is possible to obtain in vivo bioavailability/bioequivalence waivers based on in vitro dissolution for changes in formulations that usually require an in vivo study. The IVIVC guidance released by the FDA in September 1997 [9] recommends that in vivo bioequivalence studies for extended release products could be waived if the sponsor has developed a correlation whose predictability has been evaluated. In most cases, a level A correlation whose predictability has been properly evaluated is used to establish the usefulness of the in vitro dissolution as a surrogate for the bioavailability of the product under question. In this case, the waiver is granted if the difference in the predicted mean CMAX and AUC between the test and reference product is not more than 20%.

If an IVIVC is developed with the highest strength, waivers for changes made on the highest strength and any lower strengths may be granted if these strengths are compositionally proportional or qualitatively the same, the in vitro dissolution profiles of all the strengths are similar, and all strengths have the same release mechanism.

This biowaiver is applicable to new strengths lower than the highest strength, within the dosing range that has been established to be safe and effective, if the new strengths are compositionally proportional; have the same release mechanism; have similar in vitro dissolution profiles; and are manufactured using the same type of equipment and the same process at the same site as other strengths that have bioavailability data available.

For generic products to qualify for this biowaiver, one of the following situations should exist:

- Bioequivalence has been established for all strengths of the reference-listed product.
- Dose proportionality has been established for the reference-listed product, and all reference product strengths are compositionally proportional or qualitatively the same, have the same release mechanism, and the in vitro dissolution profiles of all strengths are similar.

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- Bioequivalence is established between the generic product and the reference-listed product at the highest and lowest strengths and, for the reference-listed product, all strengths are compositionally proportional or qualitatively the same, have the same release mechanism, and the in vitro dissolution profiles are similar.

To obtain a waiver for establishing bioequivalence of a new strength for a generic product, the difference in predicted means of CMAX and AUC should be no more than 10% based on dissolution profiles of the highest strength and lower strength product.

The IVIVC guidance defines the following situations where an in vivo bioavailability/bioequivalence cannot be granted even in the presence of an established IVIVC:

a. Approval of a new formulation of an approved ER drug product when the new formulation has a different release mechanism.
b. Approval of a dosage strength higher or lower than the doses that have been shown to be safe and effective in clinical trials.
c. Approval of another sponsor's MR product even with the same release-controlling mechanism.
d. Approval of a formulation change involving a nonrelease-controlling excipient in the drug product that may significantly affect drug absorption. For more detailed information on the development, evaluation and applications of IVIVC, the reader is reffered to Chapter 18 on this topic.

WAIVER OF IN VIVO BIOEQUIVALENCE BASED ON BIOPHARMACEUTICS CLASSIFICATION SYSTEM

Waiver considerations based on the BCS approach are currently applicable to IR products only. Also, BCS-based biowaivers are not applicable to "Narrow Therapeutic Range" drugs and products designed to be absorbed in the oral cavity [10].

The BCS is a scientific framework for classifying drug substances based on two fundamental properties of a drug substance, i.e., its aqueous solubility and intestinal permeability. A drug substance can have either a high- or a low-aqueous solubility and either a high- or a low-intestinal permeability. Thus, there are four BCS classes: Class 1 (High Solubility-High Permeability); Class 2 (Low Solubility-High Permeability); Class 3 (High Solubility-Low Permeability); and Class 4 (Low Solubility-Low Permeability). In addition, BCS also takes into account drug product

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dissolution, and a drug product can have either a rapid or slow dissolution. Thus, the BCS takes into account three major factors that govern the rate and extent of drug absorption from IR solid oral dosage forms: dissolution, solubility, and intestinal permeability. The central principle behind BCS-based biowaiver considerations is that when the in vivo dissolution of an IR solid oral dosage form is rapid in relation to gastric emptying and the drug has high permeability, the rate and extent of drug absorption is unlikely to be dependent on drug dissolution and/or gastrointestinal transit time. Under such circumstances, demonstration of in vivo BA or BE may not be necessary for drug products containing Class 1 drug substances that exhibit rapid in vitro dissolution, as long as the inactive ingredients used in the dosage form do not significantly affect absorption of the active ingredients.

For BCS-based waiver considerations, the drug substance should be highly soluble and highly permeable and the drug product should be rapidly dissolving. Each of these criteria is defined further below.

Solubility. The solubility class boundary is based on the highest dose strength of an IR product that is the subject of a biowaiver request. A drug substance is considered highly soluble when the highest dose strength is soluble in 250 mL or less of aqueous media over the pH range of 1–7.5.

Permeability. The permeability class boundary is based indirectly on the extent of absorption (fraction of dose absorbed, not systemic BA) of a drug substance in humans and directly on measurements of the rate of mass transfer across human intestinal membrane. Alternatively, nonhuman systems capable of predicting the extent of drug absorption in humans can be used (e.g., in vitro epithelial cell culture methods). In the absence of evidence suggesting instability in the gastrointestinal tract, a drug substance is considered to be highly permeable when the extent of absorption in humans is determined to be 90% or more of an administered dose based on mass balance determination or in comparison to an intravenous reference dose.

Dissolution: An IR product is considered rapidly dissolving when no less than 85% of the labeled amount of the drug substance dissolves within 30 minutes, using U.S. Pharmacopeia Apparatus I at 100 rpm (or Apparatus II at 50 rpm) in a volume of 900 mL or less in each of the following media: (1) 0.1 N HC1 or Simulated Gastric Fluid USP without enzymes; (2) at pH 4.5 buffer; and (3) a pH 6.8 buffer or Simulated Intestinal Fluid USP without enzymes. A sponsor/applicant can request waiver of in vivo BA and/or BE studies for IR solid dosage forms based on BCS approach during the IND, NDA, ANDA, and supplemental stages of an application. These waivers are intended to apply to subsequent in vivo BA or BE studies after initial establishment of the in vivo BA of IR dosage forms during the IND period, and in vivo BE studies of IR dosage forms in ANDAs and postapproval period.

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Once the in vivo BA of a formulation is established during the IND period, waivers of subsequent in vivo BE studies, following major changes in components, composition, and/or method of manufacture (e.g., similar to SUPAC-IR Level 3 changes) may be possible using the BCS. Biopharmaceutics classification system-based biowaivers are applicable to the to-bemarketed formulation when changes in components, composition, and/or method of manufacture occur to the clinical trial formulation, as long as the dosage forms have rapid and similar in vitro dissolution profiles. This approach is useful only when the drug substance is highly soluble and highly permeable (BCS class 1), and the formulations pre- and post-change are pharmaceutical equivalents. Biopharmaceutics classification system-based biowaivers are intended only for BE studies. They do not apply to food-effect BA studies or other pharmacokinetic studies.

For ANDAs, BCS-based biowaivers can be requested for rapidly dissolving IR products containing highly soluble and highly permeable drug substances, provided that the reference-listed drug product is also rapidly dissolving and the test product exhibits similar dissolution profiles to the reference-listed drug product. This approach is useful when the test and reference dosage forms are pharmaceutical equivalents. The choice of dissolution apparatus (USP Apparatus I or II) should be the same as that established for the reference-listed product.

Biopharmaceutics classification system-based biowaivers can be requested for significant postapproval changes (e.g., Level 3 changes in components and composition) to a rapidly dissolving IR product containing a highly soluble, highly permeable drug substance, provided that dissolution remains rapid for the postchange product and both pre and postchange products exhibit similar dissolution profiles. This approach is useful only when the drug products pre and postchange are pharmaceutical equivalents.

For additional details like methodology, etc., the reader is referred to the guidance [10]. It should also be noted that there is a great amount of research and activity currently going on in terms of whether it is possible to extend the limits of criteria by which a drug can be classified as BCS class 1 as well as whether BCS-based waivers can be extended into other BCS classes, and the reader is encouraged to keep abreast of peer-reviewed journals in the area of biopharmaceutics as the debate and discussion on BCS continues!

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JAPANESE GUIDELINES FOR IN VIVO BIOAVAILABILITY/ BIOEQUIVALENCE WAIVERS

On February 14th 2000, the Japanese regulatory health agency issued two guidances, the first entitled: "Guideline for bioequivalence studies for formulation changes of oral solid dosage forms" [11], the second entitled "Guideline for bioequivalence studies for different strengths of oral solid dosage forms" [12]. These guidelines define the levels of changes in individual excipients and categorize them into five different levels which are summarized in Table 3 and 4. When the ratios of compositions are identical between test and reference products, the formulation change is level A. This means that test and reference products are the same in ratios of all components including coating agents and, in the case of coated products, the weight of film and/or sugar-coated layers per surface area of the core must be the same. When the ratios are not identical, the levels of changes in individual excipients and categorized excipients in Tables 3 and 4 should be determined. If the change is equal to or less than the ranges of level B, it is level B. If the change is more than the ranges of level B and equal to or less than the ranges of level C, it is level C. Similarly, the change in excipients in the range between C and D is

TABLE 3 Level of Change in Individual and Categorized Excipients (Uncoated Product)

Excipient Category and component	Level		
	B	C	D
Disintegrant			
Starch	3.0	6.0	9.0
Other	1.0	2.0	3.0
Binder	0.5	1.0	1.5
Lubricant or polisher			
Ca or Mg stearate	0.25	0.50	0.75
Other	1.0	2.0	3.0
Glidant			
Talc	1.0	2.0	3.0
Other	0.10	0.20	0.30
Filler	5.0	10	15
Others[1]	1.0	2.0	3.0
Total change[2]	5.0	10	15

Figures show the percent excipient (w/w) compared to total dosage form weight.
[1]E.g., preservatives, stabilizer. Excipients of trace use are excluded.
[2]Total additive effects of all excipient changes.

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TABLE 4 Test Requirements for Each Level of Change as a Function of Therapeutic Range and Solubility for IR, DR, and CR Dosage Forms

Level	Dosage form[1]	Therapeutic range	Solubility[2]	Dissolution	Test[3]
A	—	—	—	—	A single dissolution test
B	—	—	—	—	Multiple dissolution tests
C	IR, DR	Not narrow	Not low	—	Multiple dissolution tests
	IR, DR	Not narrow	Low	—	In vivo test
	IR, DR	Narrow	Not low	⩾85%, 30 min	Multiple dissolution tests
	IR, DR	Narrow	Not low	<85%, 30 min	In vivo test
	IR, DR	Narrow	Low	—	In vivo test
	CR	Not narrow	—	—	Multiple dissolution tests
	IR, DR	Narrow	—	—	In vivo test
D	IR	Not narrow	Not low	⩾85%, 30 min	Multiple dissolution tests
	Other IR, DR, CR	—	—	—	In vivo test
E	—	—	—	—	In vivo test

[1]IR, DR, and CR mean immediate release (conventional), delayed-release (enteric coated) and controlled-release dosage forms, respectively.
[2]Products containing low solubility drugs are determined by dissolution tests. When dissolution from the reference product does not reach 85% at 2 hr at pH 1.2 and 6 hr at other pHs by paddle method at 50 rpm without surfactants, the drug is low solubility.
[3]Single and multiple dissolution tests mean the test performed under specification conditions and those under multiple conditions. When equivalence in dissolution is not shown, in vivo tests should be performed according to the guideline for bioequivalence studies of generic products.

level D. Any change in excipients whose use is limited to a trace is level A. Among the above changes, the highest level of change is defined as the level of formulation change. In the case of enteric coated products, the change in the size of the dosage form from less than 4 mm to more than 4mm or vice versa is a formulation change of level E. Depending on the level of change and the comparability of dissolution profiles in one or more dissolution medium, a bioequivalence waiver could be granted. Table 5 summarizes the regulatory requirements for each level of change.

When multiple dissolution tests are recommended or in situations where there is no approved dissolution method, the following is a description of the required dissolution tests:

Dissolution tests should be performed, using a suitably validated dissolution system and assay according to the following conditions:

1. **Number of units:** 12 units or more under each testing condition.
2. **Testing time:** 2hr in pH 1.2 medium and 6hr in other test fluids.

The test can be stopped at the time when the average dissolution of reference product reaches 85%.

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TABLE 5 Level of Change in Individual and Categorized Excipients (Coated Product)

Core/coated layer	Excipient category and component	Level B	Level C	Level D
Core	Disintegrant			
	Starch	3.0	6.0	9.0
	Other	1.0	2.0	3.0
	Binder	0.5	1.0	1.5
	Lubricant or Polishes			
	Ca or Mg stearate	0.25	0.50	0.75
	Other	1.0	2.0	3.0
	Glidant			
	Talc	1.0	2.0	3.0
	Other	0.10	0.20	0.30
	Filler	5.0	10	15
	Other[1]	1.0	2.0	3.0
	Total change[2]	5.0	10	15
Film-coated layer[3]	Total change in components[2]	5.0	10	15
	Weight of film coated layer/ surface of core[5]	10.0	20	30
Sugar-coated layer	Total change in components[2]	5.0	10	15
	Weight of sugar-coated layer/surface of core[5]	10.0	20	30

Figures show percent excipient (w/w) compared to total dosage form weight. [1]E.g., preservatives, stabilizer. Excipients of trace use are excluded.
[2]Total additive effects of all excipient changes
[3]Except for sugar-coated layer, all film-coated layers for water-proofing, undercoating, enteric coating, and controlled-release are included.
[4]Excipients of trace use are excluded
[5]The surfaces of cores are determined from the shapes of dosage forms. If it is difficult, the surface should be calculated under the assumption that the cores are spheres and the densities do not change with the formulation change.

3. **Testing conditions:** The test should be carried out under the following conditions.

Apparatus: JP paddle apparatus.
Volume of test solution: Usually 900 mL.
Temperature: 37°+/-0.5.

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Test solutions: The 1st and 2nd fluids for the disintegration test (JP13) are used as pH 1.2 and 6.8 test solutions, respectively. Diluted McIlvaine buffers (0.05 M disodium hydrogen phosphate/0.025 M citric acid) are used for other pH solutions. Other suitable test fluids can be employed when the average dissolution of the reference product does not reach 85% at 6hr in the McIlvaine buffers.

Products Containing Acidic Drugs

Agitation (rpm)	Test fluid
50	(1) pH 1.2
50	(2) pH 5.5–6.5
50	(3) pH 6.8–7.5
50	(4) water
100	(1) or (2) or (3)[a]

The test solution should be selected which provides the slowest dissolution from the reference product and gives an average of 85% dissolution or more within the testing time specified, 2hr at pH 1.2 and 6hr at other pHs. If the dissolution from the reference product does not reach 85% at the specified time in any test fluids, the test solution providing the fastest dissolution should be used.

Products Containing Neutral or Basic Drugs, and Coated Products

Agitation (rpm)	Test fluid
50	(1) pH 1.2
50	(2) pH 3–5
50	(3) pH 6.8[a]
50	(4) water
100	(1) or (2) or (3)[a]

The test solution should be selected which provides the slowest dissolution from the reference product and gives an average of 85% dissolution or more within the testing time specified, 2hr at pH 1.2 and 6hr at other pHs. If the dissolution from the reference product does not reach 85% at the specified time in any test fluids, the test solution providing the fastest dissolution should be used.

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Products Containing Low Solubility Drugs

When the average dissolution from reference product does not reach 85% at the testing time specified (2hr at pH 1.2 and 6hr at other pHs) at 50rpm in any of the test fluids, without surfactants, employed in the above dissolution tests (1) and (2), they are defined as products containing low solubility drugs.

Agitation (rpm)	Test fluid
50	(1) pH 1.2
50	(2) pH 4
50	(3) pH 6.8
50	(4) water
50	(1) pH 1.2 + polysorbate 80
50	(2) pH 4 + polysorbate 80
50	(2) pH 6.8 + polysorbate 80
50	(3) water + polysorbate 80
100	(5) or (6) or (7)

Among 0.01, 0.1, 0.5, and 1.0 w/w% of polysorbate 80, the lowest surfactant concentration should be chosen, which provides an average of 85% dissolution or more at the testing time specified (2 hr at pH 1.2 and 6 hr at other pHs) in at least, one of the test fluids. Dissolution tests in the four fluids should be performed at the same surfactant concentration chosen. If the average dissolution from the reference product does not reach 85% at the specified time in any of test fluids, the surfactant concentration providing the fastest dissolution should be selected. Among the three test solutions, the testing fluid providing the slowest dissolution from the reference product and giving an average 85% dissolution or more within the testing time specified should be selected. If the average dissolution from the reference product does not reach 85% at the specified time in any of the test fluids, the test solution providing the fastest dissolution should be used.

Enteric Coated Products

Agitation (rpm)	Test fluid
50	(1) pH 1.2
50	(2) pH 4
50	(3) pH 6.8
50	(4) water
100	pH 6

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Enteric coated products containing low solubility drugs should be tested by adding polysorbate 80 to the test fluids (2) and (3) according to the dissolution test for products containing low solubility drugs as described above.

Acceptance Criteria for Equivalence of Dissolution Profiles

The acceptance criteria for equivalence of dissolution profiles is based both on average and individual dissolution profiles. Test and reference products are considered equivalent when they meet both requirements (1) and (2) shown below. The rule is not applicable to conventional dosage forms and enteric coated products, unless the average dissolution from the reference product reaches 85% under any of the testing conditions. If a dissolution lag is observed for reference products, the equivalence in dissolution can be assessed using the dissolution profile normalized for the lag time (see below).

Average Dissolution

a. *When the average dissolution from the reference product reaches 85% within 15min:* The average dissolution from the test product also reaches 85% within 15min or does not deviate by more than 10% from that of the reference product at 15min.

b. *When the average dissolution from the reference product reaches 85% between 15 and 30min:* The average dissolution from the test product does not deviate by more than 10% from that of the reference product at two time points where the average amounts dissolved from the reference product are around 60 and 85%. When f_2 is used, the f_2 value should not be less than 50.

c. *When the average dissolution from the reference product does not reach 85% in 30min:* The following criteria should be applied to the comparison of average dissolution profiles (2hr at pH 1.2 and 6hr at other pHs for conventional and enteric coated products and 24 hr for controlled-release products).

When the dissolution profiles are normalized for the lag time, the difference in average lag time between test and reference products should not be more than 10min.

d. *When the average dissolution from the reference product does not reach 50% at the testing time point:* The average dissolution of test product does not deviate by more than 6% from that of the reference product at the time points specified, or the f_2 value is equal to or more than 60.

When the average dissolution from the reference product is between 50 and 85% at the testing time point:

The average dissolution of the test product does not deviate by more than

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8% from that of the reference product at the time points specified, or the f_2 value is equal to or more than 55.

e. *When the average dissolution from the reference product reaches 85% within the testing time:* the average dissolution from the test product does not deviate by more than 10% from that of the reference product at the time points specified, or the f_2 value is equal to or more than 50.

Individual Dissolution

Test products (n=12) should meet one of the following requirements at the final time points where the average dissolution is compared between test and reference products.

a. *When the average dissolution of the reference product does not reach 50% within the testing time:* There is no sample of test products that shows the deviation of more than 15% in dissolution from the average dissolution of the reference product, and one or no sample that shows the deviation of more than 10%.

b. *When the average dissolution of the reference product is between 50 and 85% at the testing time point:* There is no sample of test product that shows a deviation of more than 20% in dissolution from the average dissolution of the reference product, and one or no sample that shows a deviation of more than 12%.

c. *When the average dissolution of the reference product reaches 85% within the testing time:* There is no sample of test product that shows a deviation of more than 25% in dissolution from the average dissolution of the reference product, and one or no sample that shows a deviation of more than 15%.

Time Point for f_2

a. *When the average dissolution from the reference product reaches 85% between 15 and 30min:* 15, 30, and 45min.

b. *When the average dissolution from the reference product reaches 85% between 30 min and the testing time point:* Ta/4, 2Ta/4, 3Ta/4, and Ta, where Ta is the time point at which average dissolution from the reference product reaches approximately 85%.

c. *When the average dissolution from the reference product does not reach 85% at the testing time point:* Ta/4, 2Ta/4, 3Ta/4, and Ta, where Ta is the time point at which average dissolution from the reference product reaches approximately 85% of the final amount dissolved in the testing time.

When there is a lag in dissolution, dissolution data normalized for the lag time should be used for the calculation of f_2.

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Normalization of Dissolution Profiles with Lag Time

The lag time is conventionally defined as the time when 5% of the drug dissolves. The lag time should be determined for individual dissolution by linear interpolation, followed by normalization of dissolution profiles for the lag time. Then, the average dissolution profiles are determined which can be used for the assessment of equivalence in average dissolution.

EUROPEAN GUIDANCE FOR AN IN VIVO BIOAVAILABILITY BIOEQUIVALENCE WAIVERS

According to the European Agency for the Evaluation of Medicinal Products guidance on the investigation of bioavailability and bioequivalence [14] if a new application concerns several strengths of the active substance, a bioequivalence study investigating only one strength may be acceptable. However, the choice of the strength used should be justified on analytical, pharmacokinetic, and safety grounds. Furthermore, all of the following conditions should be fulfilled:

- The pharmaceutical products are manufactured by the same manufacturer and process.
- The drug input has been shown to be linear over the therapeutic dose range (if this is not the case the strengths where the sensitivity is largest to identify differences in the two products should be listed).
- The qualitative composition of the different strengths is the same.
- The ratio between amounts of active substance and excipients is the same, or, in the case of preparations containing a low concentration of the active substance (less than 5%), the ratio between the amounts of excipients is similar.
- The dissolution profile should be similar under identical conditions for the additional strengths and the strength of the batch used in the bioequivalency study.

If a new strength (within the approved dose range) is applied for on the basis of an already-approved medicinal product and all of the stated conditions hold then a bioequivalence study is not necessary.

In case of exemption from bioequivalence studies, in vitro data should demonstrate the similarity of dissolution profile between the test product and the reference product in each of the three buffers within the range of pH 1–8 at 37°C (preferably at or about pH 1, 4.6, and 6.8). This is done using the f_2 similarity factor. However, in cases where more than 85% of the active

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substance is dissolved within 15 min, the similarity of dissolution profiles may be accepted without any mathematical evaluation.

CANADIAN GUIDANCE FOR IN VIVO BIOAVAILABILITY/ BIOEQUIVALENCE WAIVERS

According to the Drug Directorate of Canada guideline on the conduct and analysis of bioavailability and bioequivalence studies for uncomplicated drugs in which the proportions of excipients to the drug and the dissolution characteristics are the same, it is sufficient to establish the bioavailability of one strength. Whether all strengths of other products should be tested will depend on the extent to which the other formulations differ in strength. For some of the complicated drugs such as those with narrow therapeutic range, steep dose response characteristics, or nonlinear kinetics, a single-dose bioavailability study should be conducted on each strength [15].

CONCLUSION

More and more regulatory agencies around the world are relying on in vitro dissolution to assess the bioavailability and the bioequivalence of drug products. The dissolution test is no longer looked at as only a quality control tool but also as an indicator of the bioavailability of a drug product. Minor formulation changes can be approved just based on dissolution data and even major formulation changes that required bioequivalence studies in the past are being waived if the drug belonges to BCS class I or if there is a predictive IVIVC. Thus dissolution testing if done properly can result in decreasing the regulatory burden on sponsors by decreasing the number of in vivo studies that are needed to approve and maintain a drug product on the market. That is why during the development stage of a drug, proper care and attention should be paid to develop the most appropriate dissolution method that is discriminatory and that will as much as possible have the ability to reject formulations or lots with an inadequate in vivo bioavailability profile.

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Bioavailability and Bioequivalence Issues for Drugs Administered via Different Routes of Administration; Inhalation/Nasal Products; Dermatological Products, Suppositories

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OVERVIEW

While oral dosage forms represent the preferred route of drug delivery, there are situations when nonoral routes are indicated. In this chapter, we will present an overview of the issues involved in assessing bioavailability and bioequivalence via nonoral routes of administration. Each route of administration will be presented individually along with a discussion of some of the pharmaceutic and physiologic factors affecting drug absorption. Examples of how some of these factors can interplay in the design and evaluation of these dosage forms will also be presented.

INTRODUCTION

While oral dosage forms are the primary route of delivery of most Pharmaceuticals there are times where either due to pharmacokinetic factors

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(such as first-pass metabolism), or due to a desire to minimize systemic effects through local administration, the disease state itself (i.e., extreme nausea and vomiting) will not allow for oral dosing. In these situations, alternative routes of administration must be utilized to obtain the desired therapeutic outcome. Consequently, in the development of drugs for these routes of delivery great care must be taken to consider the unique challenges that each of these routes presents in relation to bioavailability and bioequivalence testing.

The nasal, dermatological, and rectal routes of administration, although on the surface are quite distinct, they are all linked in that they are all, in a broad sense, examples of topical drug application but not necessarily topical drug delivery. The difference is that in topical drug delivery the drug is administered for a local effect, as is most often the case in applying drugs to the skin. In comparison, both the intranasal and rectal routes are often chosen to provide systemic drug delivery under special circumstances.

Inherent in these three routes of administration is the fact that all of them are not normally thought of as being naturally permeable to drug absorption to any great extent. For example, the skin is first and foremost a barrier protecting internal tissues from external insult, be they chemical, bacterial, or physical in nature. Likewise, in the nose, the nasal passages and structural components are present not for drug absorption but to act as a filter to remove inhaled pollen, bacteria, and other suspended particulates prior to their delivery to the lung. The rectum, while the distal end of the digestive tract, does not have the structure of the small intestine or the enzymes and digestive juices present to enhance nutrient absorption. Because none of these tissues are inherently designed for drug/nutrient absorption choosing them as a site for drug delivery requires an assessment of physiochemical properties of the drug, the target tissues, and the performance mechanics of the drug delivery device/vehicle.

INHALATION/NASAL DRUG PRODUCTS

For the most part, the application of drug substances to the nasal mucosa has historically been limited to topically acting agents for the symptomatic treatment of allergic rhinitis and the common cold. In the last ten to fifteen years a renewed interested in the nasal route of drug delivery has occurred as a method of delivering protein-based therapeutic agents that would be unstable in the gastric/digestive environment. The archetypical drug that has been proposed in the literature is insulin. Insulin given by the intranasal route would provide a quicker onset of action, relative to subcutaneous use, and would be more physiologic in its action. The delivery characteristics of insulin and other small protein-based drug products such as vaccines via the

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intranasal route are a route of great promise for the small bioactive molecules and are actively being pursued.

Anatomy and Physisology of the Nose

Although the external nasal tissue differs markedly in size and shape from individual to individual, regardless of its external size, it is the large internal surface area of the nose, which helps it perform its many functions as both sensory and respiratory organ (Fig. 1). While the shape of the external nasal tissue, the "nose" itself, may in severe instances restrict airflow, this does not routinely play a role in the delivery of drug to the nasal tissues due to placement of the pump/spray unit within the nasal cavity. The barriers to drug absorption in the nose can be classified as mechanical (cilia function), passive entrapment (mucous production), and enzymatic (nasal P-450 activity).

As a filter, the nasal mucosa prevents the entry of particles larger than 5 μm in diameter, and most smaller particles into the lower respiratory tract.

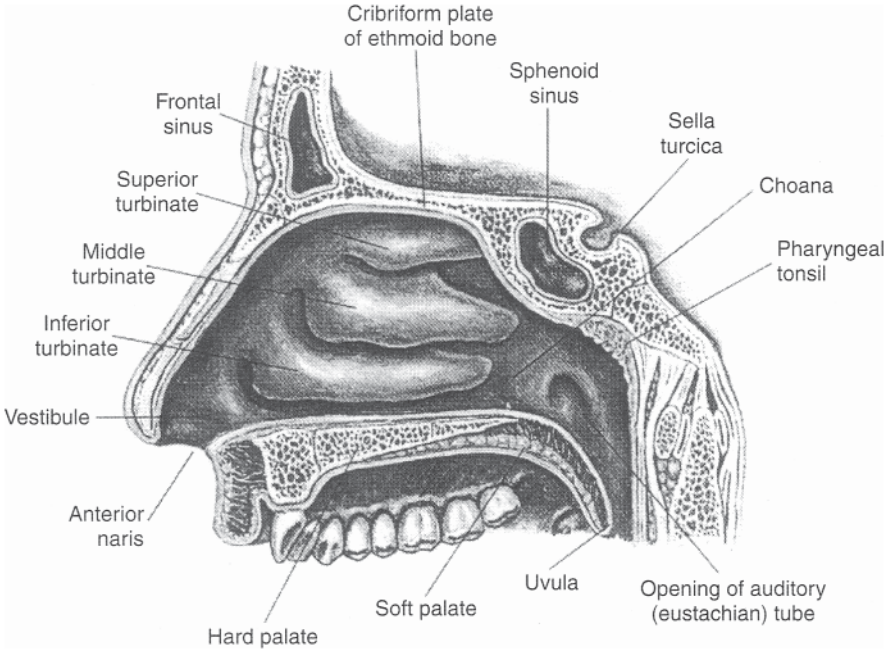

FIGURE 1 The internal surface area of the nose. *Source:* Ref. 60, p. 312.

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In addition, the nose through its extensive vascular supply rapidly, but only partially, regulates the temperature and humidity of inhaled air (~ 10,000 L per day) despite changes in external air temperature that can rapidly change from a heated room to subzero conditions. The ability of the nose to filter particles efficiently from inspired air is accomplished by several mechanisms. A large proportion of inhaled particulate matter is deposited at the anterior unciliated area of the nasal passages as a direct result of filtration by nasal vestibule (i.e., the external nasal tissues). The nasal valve at the posterior end of the vestibule limits the rate of inspiratory nasal air flow and accounts for ~50% of the total resistance to airflow from ambient air to the alveoli. Internally, the nasal turbinates increase the mucosal surface area of the nasal cavity to approximately 100 to 200cm^2 and regulate airflow by changing the blood content of the highly vascular turbinates both spontaneously and rhythmically (i.e., the "nasal cycle"). The turbulence of the air passing through the nose also helps cause impaction of particles and assists all the other functions of the nose. These cyclic changes in resistance to airflow occur in 80% of normal subjects; each nasal cycle lasts from two to six hours. As airway resistance increases in one nostril, it decreases in the other.

Inspired particles are further filtered by their entrapment of inhaled particles in a mucous "blanket", on the surface of the ciliary epithelium approximately 10 to 15 μm deep. This mucous "blanket" starts posterior to the anterior tip of the inferior nasal turbinate and covers the entire nasal cavity. It is a watery mixture consisting primarily of proteins, six of which are derived from plasma. The mucus is secreted by surface goblet cells that line the nasal cavity. The principal protein and antibody present is immunoglobulin A (IgA), which is synthesized against viral respiratory infection antigens as well as other antigens. Besides this antigen antibody response the mucous provides a physical barrier and effectively traps and removes particles greater than 4μm in diameter. Mucociliary transport moves the blanket, with its contents, posteriorly toward the nasopharynx at an average rate of 8 to 9 mL per min, except at the anterior portion of the inferior turbinates where it moves anteriorly. Throughout the day the normal pH of the nasal cavity varies between 5.97 and 7.85 and is markedly constant showing no change in response to rest or meals. Ideally, it is into this milieu that inspired drug particles are trapped and become solubilized for delivery to the nasal tissues for absorption. In contrast drug particles that become directly lodged in the cilia are rapidly cleared under normal circumstances.

Environmental irritants such as tobacco smoke may significantly decrease the ciliary activity of the nasal mucosa. If destroyed as a result of infection, the epithelium can regenerate, although such regeneration may take from a few hours to two weeks postinfection depending on the depth and scope of the insult. Occasionally, following either a massive acute insult or the result

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of a chronic disease process, the nasal mucosa does not regenerate. In such cases, the filtering ability of the nose is greatly decreased and larger particles are allowed to penetrate deeper into the respiratory tract. Normally clearance of particles from the nasal mucosa occurs within 15min of deposition by the combined effects of mucous trapping and ciliary action. This "residence time" in the nasal mucosa can be increased into hours by the in situ formation of a bioadhesive or "mucoadhesive" delivery system, allowing for localization of drug and enhancement of drug delivery. This has implications for drug delivery where particle size control of droplet formation is critical to targeted drug delivery.

Drugs for Nasal Delivery

As mentioned previously the primary use of drugs administered intranasally has been to treat allergic rhinitis and the common cold. This includes agents such as the topical corticosteroid (betamethasone, fluticasone, budesonide, etc.) topical vasoconstrictors (oxymetazoline, phenylephrine, etc.) and other miscellaneous agents such as cromolyn sodium. All of these agents work to improve airflow from the nasal mucosa by either dilating the nasal passages (Fig. 1) or decreasing the immune response via local mechanisms of action. As such bioavailability/bioequivalence testing of such compounds is limited by the small doses administered and the biological response.

Systemic drugs such as intranasal butorphanol (Stadol NS) and nicotine (Nicotrol nasal spray) are delivered intranasally to either avoid first-pass metabolism or provide effective drug levels rapidly. In the case of nicotine, comparative in vivo bioavailability studies comparing the intranasal spray to other routes of administration clearly shows that it produces plasma levels inferior to those of a cigarette, but superior in rate to most other routes of administration. Thus the intranasal spray form of nicotine provides rapid vascular access to the brain without coadministration of the accompanying carcinogens formed from the burning of tobacco. Used as a part of a smoking cessation program the nasal spray can be effective in lessening and then elimination of the addiction.

Bioavailability/Bioequivalence Considerations

General Considerations

For both systemically delivered agents and agents for topical treatment, the following table summarizes some of the considerations which must be taken into consideration in the design of a nasal dosage form and its proper evaluation.

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TABLE 1 Factors for Consideration in Designing a Nasal Dosage Form

1. Drug substance properties
   a. Solubility
   b. pH
   c. Particle size (for powders)
2. Formulation issues
   a. Adjuvant agents
      – surfactants
      – permeation enhancers
3. Drug delivery
   a. Pressurized canister/spray pump
      – reproducibility of dosing, i.e. volume per spray
      – particle size distribution (for suspensions/solutions)
      – proper administration technique
4. Disease impact
   a. Rhinitis
   b. Use of decongestants/vasoconstrictive agents

Inspection of this list reveals that many of these issues relate to the development of the dosage form itself, i.e., chemistry and manufacturing considerations rather than drug absorption. Only in nasal or inhalational drug delivery does the delivery system itself play such a key role in the biopharmaceutics of a drug. This is because with inhalational drug delivery we are dispersing drug into the nasal passageways as suspended particles or droplets that then must settle out in the appropriate location, relative to the various elimination mechanisms present in the nasal cavity, for absorption to become possible. In the assessment of nasal bioavailability/bioequivalence, we must first consider whether or not the drug is intended for systemic or topical action.

Topically Acting Drugs

With topically acting drugs, such as vasoconstrictors, in vivo determination of systemic plasma levels is often impossible due to analytical constraints. In such situations, use of pharmacodynamic endpoints such vital capacity and forced expiration volume (FEV1) can be used as a surrogate measure of bioavailability. In the case of corticosteroids, the assessment of the hypothalamic-pituitary adrenal (HPA) axis suppression has been used as a systemic marker of bioavailability/bioequivalence, even though the intended

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site of action is local. In this latter case, it is the absence of effect on the HPA axis that is demonstrative of localization of drug delivery to nasal tissues.

Prior to accepting such data for a new chemical entity or even a known substance in a new formulation, an attempt should be made to first quantify the in vivo plasma levels under maximal dosing conditions. With maximal dosing conditions being defined as multiple dosing at the highest clinically tested dose and dosing frequency. This is necessary as with new agents their degree of absorption cannot be determined reliably by animal extrapolation, and in the case of older known agents, developments in both delivery system technology and analytical methodology may have reached the point of producing systemic levels. Such in vivo pharmacokinetic trials need not incorporate a large number of blood samples under the concept of a surveillance pharmacokinetics sampling strategy. This sampling strategy differs from standard geometric sampling in that it focuses the samples in the time period within which blood levels would likely occur. That is to say, with nasal products, given the mucocilliary elimination mechanisms present, drug absorption from the nasal mucosa, from immediate release products, beyond two to three hours is highly unlikely. Under a geometric sampling strategy, which would space blood samples throughout the dosing interval, numerous blood samples would essentially be wasted, adding to the inconvenience of the subject and cost of the trial. By taking blood samples only during those time periods when absorption would be expected to occur, one can reduce both the inconvenience and the cost of the trial. The down side to surveillance pharmacokinetics is that if significant and prolonged drug levels are seen, the sampling strategy may not be sufficient to determine the underlying pharmacokinetic systems. In practice, incorporating surveillance pharmacokinetic sampling into an early phase II trial can minimize this potential with a limited number of subjects. In either case, given the recent advances an analytical technology over the last decade, more and more agents that have in vivo pharmacodynamic/clinical efficacy assess-ments for bioavilability testing will be replaced with in vivo pharmacokinetic methods.

Systemically Active Agents

For those agents administered via the intranasal route for systemic effects the performance/absorption of drug from the intranasal route should be compared to that from another route of administration, be it oral or ideally intravenous. Figure 2 shows the comparative in vivo bioavailability of transnasal butorphanol relative to IV and sublingual administration, while Fig. 3 shows the comparison of the nicotine nasal spray to other routes including cigarettes. From both of these examples, the rapid nature of intranasal absorption can be seen. In the case of butorphanol, its use as a

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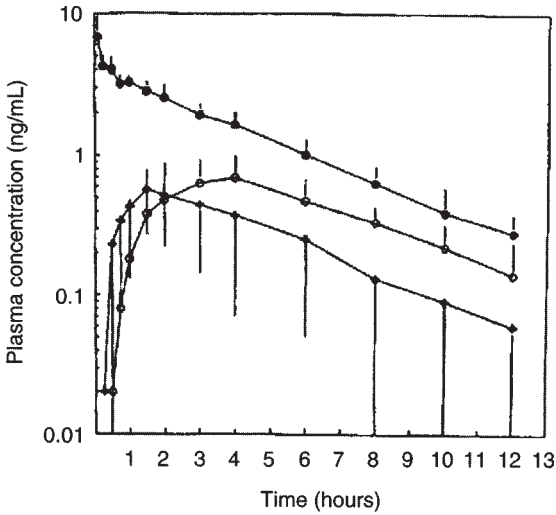

FIGURE 2 In vivo bioavailability of transnasal butorphanol relative to IV and sublingual administration. *Source:* Ref. 21, p. 376.

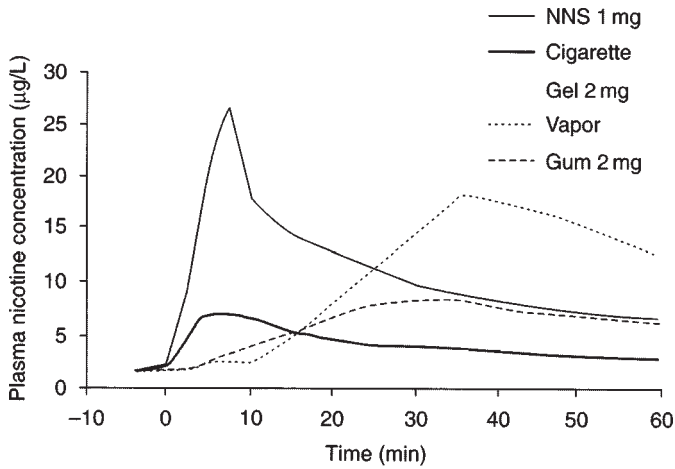

FIGURE 3 Comparison of the nicotine nasal spray to other routes including cigarettes. *Source:* Ref. 19, p. 76.

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treatment for the pain of migraine headache would require a rapid onset of action, compared to the IV formulation, the nasal spray has an absolute bioavailability of ~50%. With this information proper dose-ranging and treatment regimens can be designed and tested to maximize the attainment of effective levels for analgesia.

As for the nicotine nasal spray, Fig. 3 shows that across a number of different studies only the "gel" and nasal spray dosage forms show a rapid increase in venous levels of nicotine compared to the gum or vapor form (an early of the nicotine inhaler). Arterial levels of nicotine (Fig. 4) show that the nasal spray can achieve arterial levels rapidly and thus respond more readily to nicotine "craving" by subjects needing the rapid "hit" associated with cigarettes that is lacking with the other formulations. By understanding the need to provide quitting smokers with a nicotine delivery system that can, albeit at a reduced level, provide a cigarette like rush of nicotine levels, the relatively low rates of smoking cessation using nicotine replacement products may be increased by responding to the needs and pattern of addiction and addictive behavior.

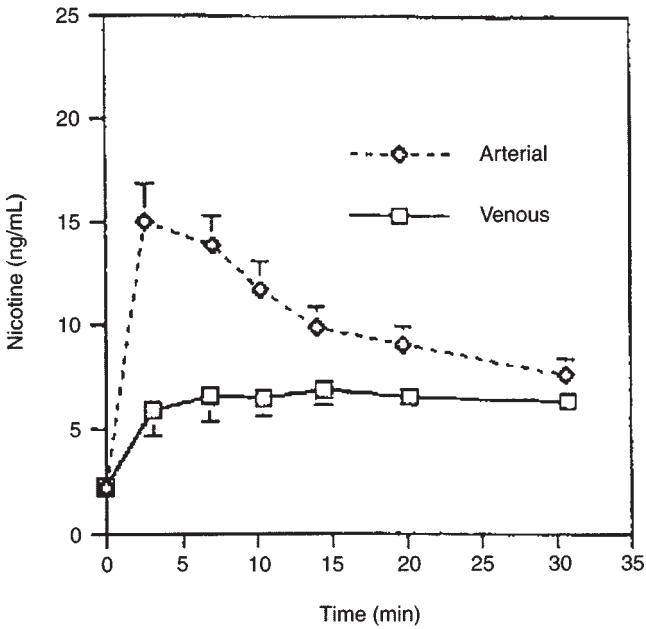

FIGURE 4 Arterial versus venous levels of nicotine over time. *Source:* Ref. 7, p. 641.

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From a formulation design aspect, studies should also be undertaken to assess the impact of multiple actuation on bioavailability. In vitro and in vivo studies have shown that when an insufficient amount of time has elapsed between actuations, the suspended drug particles in the nasal tissues often coalesce into larger particles that are more easily cleared by the nose. In doing so the resulting in vivo bioavailability of the drug can drop relative to the administered dose. This can result in an urge in the patient to increase the dose, resulting in a further loss of bioavailability and can result in reports of patient dissatisfaction with the product—a situation that proper study and patient counseling by the physician and pharmacist can overcome.

Disease State

As these products are being administered for systemic effects, consideration must be given to the impact of other disease states on drug absorption. Specifically, the effect of allergic rhinitis with its attendant copious nasal discharge should be evaluated along with the impact of topical vasoconstrictors on drug absorption. In both the situations, the impact on drug absorption needs to be determined so that the dose and or dosing instructions can be altered to maintain effective in vivo plasma concentrations. In Fig. 5, the results of a comparative in vivo bioavailability study in which smokers were given the nicotine nasal spray both in the absence of a cold and in the presence of a cold with xylometazoline. Clearly the peak plasma levels are blunted and the time to achieve these plasma levels is increased from a disease-free baseline of 0.28 to 0.40 hr with rhinitis alone, and to 0.52hr with rhinitis/xylometazoline. In a situation like nicotine replacement therapy or in the case of butorphanol, when pain relief is the endpoint, the existence of rhinitis, with or without concomitant use of a topical vasoconstrictor, can significantly affect the onset and quality of drug effect. These factors need to be considered in drug development along with strategies, for either dosing increases, or rescue/alternative treatment regimens during the time course of the cold.

Structural defects in the nose, be it a deviated nasal septum or other structural abnormality in the nasal passage can also affect the bioavailability of nasally administered drugs. However, the wide variety and severity of these defects are such that a systematic study of them prior to drug approval is not feasible. Labeling should be developed with this in mind to instruct the prescriber to consider this potentiality in selecting patients for intranasal drug delivery.

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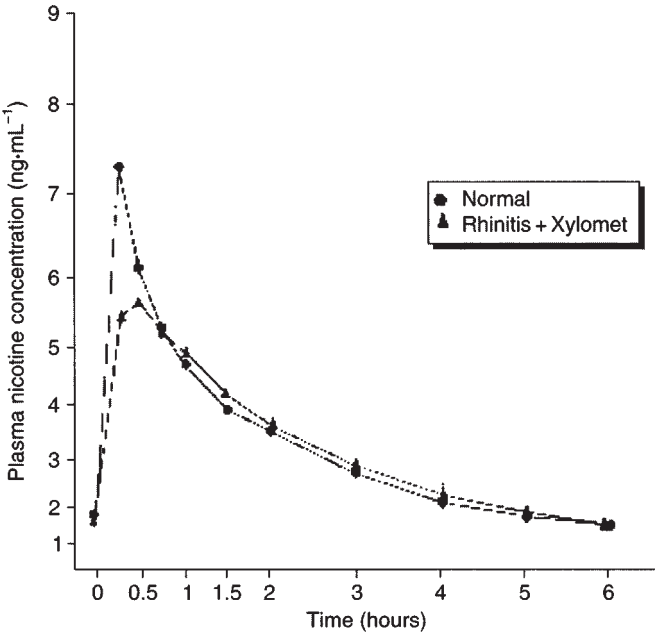

FIGURE 5 Results of a comparative in vivo bioavailability study in which smokers were given the nicotine nasal spray both in the absence of a cold and in the presence of a cold with xylometazoline. *Source:* Ref. 14, p. 73.

Delivery System

Unique to the intranasal (and other inhalational routes of drug delivery) is that additional studies may be required to assess the performance characteristics of the delivery system itself. That is the reproducibility of the pump/device to delivery a consistent dose from first to last, both in the amount of drug delivered and the production of the proper-sized particles. When possible, absolute in vivo bioavailability studies should be undertaken to determine the efficiency of the interaction between the drug-formulationroute of delivery factors previously outlined in Table 1. The data from such studies should be used to optimize the formulation in terms of delivery by modification of the particle size and spray pattern produced by the nozzle at the point of delivery.

Dosing Instructions

Prior to the use of a nasal inhaler/spray device the subject should, in turn, clear each nostril by blowing. In the case of rhinitis, the subject may wish to

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use a topical vasoconstrictor 20–30 min prior to dosing. The inhaler/spray device should be placed into the nose and with the contralateral side of the nose occluded the dose should be delivered in time with a natural intake of air. The breath should then be held for 15–20 seconds to allow time for the drug particles to settle out and become available for absorption. Then the contralateral side of the nose should be dosed according to directions, or in the case where instructions are not given, two to five min after the first dose. By providing a delay between doses, the potential for suspended droplets to coalesce into larger particles, which are more readily eliminated, is minimized.

TOPICAL DRUG DELIVERY

Topical drug delivery differs from transdermal drug delivery in that the sites of drug application and drug action are one and the same. In topical drug delivery, we are primarily concerned with delivering drug to skin itself whereas with transdermal drug delivery we are concerned with the delivery of drug through the skin to the systemic circulation. For a topically applied agent, drug that reaches the systemic circulation is essentially lost to the site of action and can result in undesired side effects. Examples of such side effects include suppression of the hypothalamic-pituitary adrenal (HPA) axis in the case of topically applied corticosteroids or birth defects in the case of topical retinoids. In this section, we will focus on the biopharmaceutic issues surrounding topical drug application.

Anatomy and Physiology of the Skin

Prior to discussing the evaluation of topical drug delivery we must first consider the skin and the various physiologic factors that affect it. The skin is the largest organ in the body with a surface area in the adult male approximating $1.73 m^2$. It is a multifunction organ which besides its structural role as a physical protective covering has important roles in thermoregulation and maintaining fluid balance. It is the first line of defense against bacterial infection and is undergoing continual replacement via the shedding of skin cells.

The skin itself is organized into discrete layers that each have a role to play in the structure and function of the skin. The outermost layer of the skin is the stratum corneum. This layer, approximately 10–15 cells thick is composed of dead skin cells (corneocytes) arranged in a so-called brick and mortar pattern with lipids representing the mortar. It is devoid of blood vessels and represents the primary barrier to the permeation of water and drug delivery. In Fig. 6, the stratum corneum is shown as the outer layer of the epidermis which can be further subdivided into the stratum granulosum

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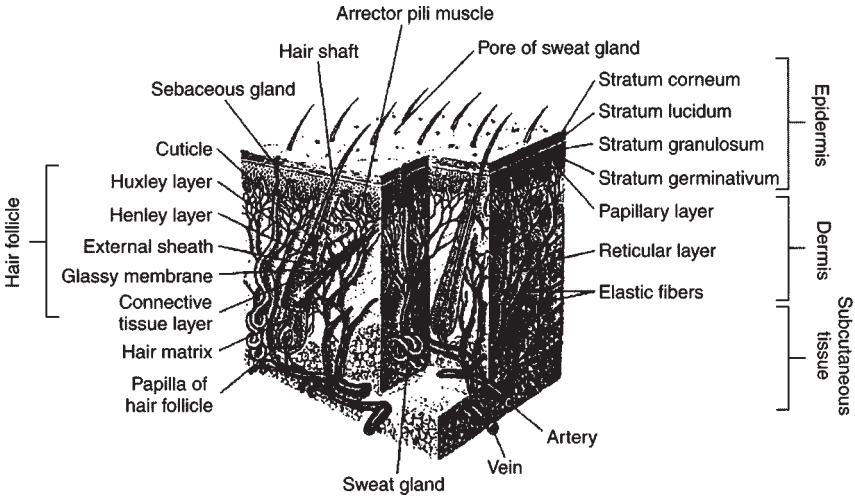

FIGURE 6 Layers of the epidermis. *Source:* Ref 60, p. 162.

and germinativum, and in the case of the thicker skin on soles of the feet and hands, the stratum lucidum. The epidermis itself lacks a system of vascular structures and is nourished by papillary capillaries in the dermis that extend upward into finger-like projections of the dermis, called dermal papillare, into the epidermis. In addition to the vascular supply for the epidermis, the dermis also contains the elastin and collagen fibers that give skin its strength and resilience along with sensory nerve fibers for pain, touch, and temperature. Most topically treated diseases are thought to arise from the upper stratum granulosum (i.e., fungal infection) to the dermis (i.e., atopic dermatitis).

Systemic drug absorption following topical application can occur via a number of mechanisms:

1. Direct absorption through the stratum corneum and epidermis to the underlying capillaries,
2. Transfollicular drug delivery via the hair shaft.
3. Drug absorption through the eccrine (sweat) gland pathway.

Of these pathways, the transfollicular and eccrine glands represent potential shunts of drug delivery that increase in importance, in normal skin, when the stratum corneum is intact. In the setting of topical drug delivery, where the stratum corneum is disrupted, these routes play a lesser role. Transfollicular absorption can become a major route for absorption in those

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situations where the site of drug action is the hair shaft itself. In the case of pediculosis (lice), topical products are often formulated as a shampoo or mousse to enhance the coating of the hair shaft. Drug is then carried down to the follicle where it can be absorbed. Because of their lipophilic nature, transfollicular absorption is thought to be a major route of pesticide absorption in field workers.

Drugs for Topical Drug Delivery

As mentioned above topical drug delivery is designed to provide local treatment to the skin and related tissues. This can be in response to a number of diseases including acne vulgaris, actinic keratosis, atopic dermatitis, psoriasis, fungal infection, and vertilligo to name but a few of many such diseases. This represents a wide range of potential disease states and their attendant treatments from antibiotics (erythromycin, clindamycin, etc.) for acne, antifungals (terbinafine, ketoconazole, etc.) for athlete's foot, retinoids (retinoic acid, tazarotene, etc.) for psoriasis and corticosteroids (betamethasone, clobetasol, etc.) for atopic dermatitis.

As in the case of intranasal drug delivery of locally acting drug products the availability of a validated analytical method will determine the types of in vivo bioavailability trials conducted. In addition to the drug, the vehicle often plays an important part in the case of localizing drug to the target site. Topical vehicles include creams, ointments, gels, solutions, lotions, mousse, shampoos, foam, and variations on these themes. While it is tempting to generalize that all lotions are more available than creams and ointments, this is not always the case. With topically applied drugs absorption is dependent on the interplay between the skin, vehicle, drug, and any permeation enhancers that may be present in the formulation.

Bioavailability/Bioequivalence Considerations

General Design Factors

In most diseases of the skin, the structural layers and/or integrity of the skin are disrupted, and drug penetration throughout the stratum corneum to the other layers of the epidermis and dermis are altered. It is for this reason that in vivo bioavailability studies should always be conducted in the target patient population with disease severity approximating the upper limit of that allowed for in the planned clinical development program. In this case, the use of healthy normal volunteers is of no value in assessing the pharmacokinetics of drug absorption in diseased skin. The only exception to this general rule would be in the case of diseases of pigmentation (both hyper- and hypo-) such as vitiligo in which case the underlying structure of the skin is unchanged.

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In such situations as this, the use of normal subjects or areas of nondiseased skin in subjects with the disease is allowable. However, individual study guidance from the regulatory body in question should be sought to obtain an agreement on this and other study design issues prior to study initiation.

Similar to the concepts used in the evaluation of intranasal dosage forms, the underlying principle of pharmacokinetic study design in topical products is to maximize the potential for systemic levels to occur. This is done by modifying those factors that affect topical drug absorption, see Table 2.

By maximizing these elements in the setting of diseased skin one can often produce systemic plasma levels, or in the case of corticosteroids, produce clinically significant HP A axis suppression. Given the chronic nature of many topical diseases, such as atopic dermatitis and psoriasis, systemic availability and its assessment is critical to the overall safety determination for a drug.

An example of the kind of plasma levels that can be achieved with topical dosing under chronic conditions is that of tazarotene. When applied to normal (i.e., non-diseased) skin, the systemic absorption of tazarotene is low (~1%) even after multiple dosing. However, in three-month study of subjects with psoriasis, with a mean total body area involvement of 13%, systemic levels of tazarotenic acid (the active metabolite) were detectable with an estimated bioavailability, upon multiple-dosing, of <5% (Fig. 7). Interestingly, with continued-dosing, the bioavailability of tazarotene drops over time until it approaches that of healthy individuals. This is thought to be due to the retinoid effects on clearing the psoriatic plaques allowing the re-establishment of an effective skin barrier, and thus decreasing the permeability of the skin to tazarotene.

In vitro Methods

As mentioned earlier, when a sponsor is pursuing the development of multiple topical formulations, an in vivo biostudy with the most bioavailable dosage

TABLE 2 Topical Bioavailability Study Design Elements

Involved Surface Area (diseased skin)
Amount of Drug Product Applied
Proposed Concentration
Frequency of Application
Duration to Allow Attainment of Steady-State Levels

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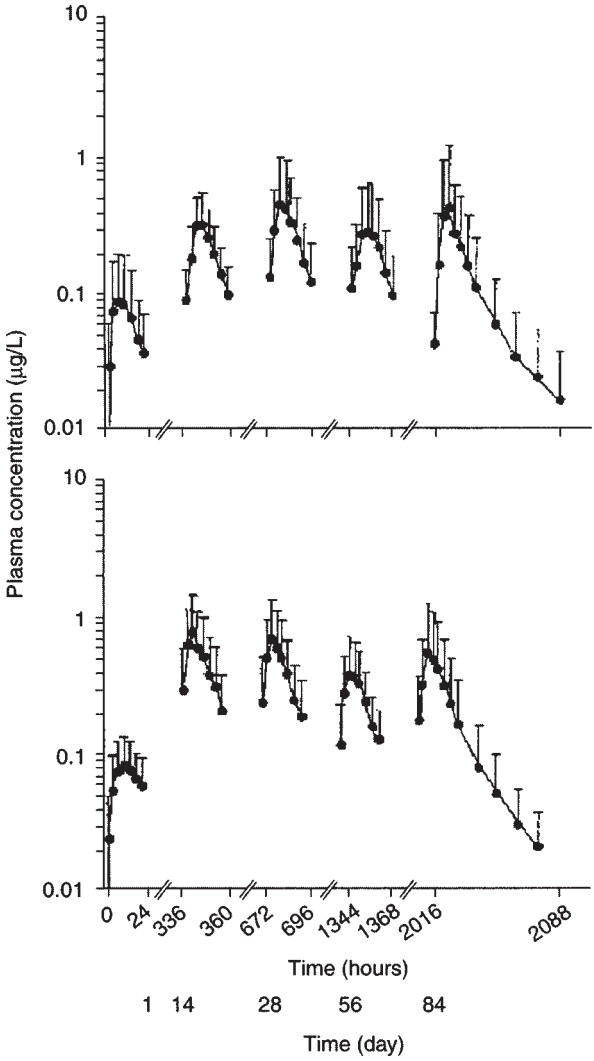

FIGURE 7 A three-month study of subjects with psoriasis, with a mean total body area involvement of 13%, systemic levels of tazarotenic acid (the active metabolite) were detectable with an estimated bioavailability, upon multipledosing, of <5%. *Source:* Ref. 37, p. 280.

form may be sufficient under certain situations. Unfortunately, while in vitro methods, using such apparatus as the Franz Diffusion Cell may be useful for assessing the relative penetration of drug through intact skin. The relationship

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between the degree of drug penetration of both diseased and normal skin varies from disease to disease and within a disease according to severity and/or extent of involvement. This basic alteration in skin structure severely limits the utility of in vitro and novel in vivo test methods in the evaluation of topical dosage forms. While they may be useful in the initial screening of topical formulations in healthy adults or through the use of cadaver skin, such methods as diffusion cells, tape stripping and microdialysis all share these same limitations.

Age

Another element to be considered in the evaluation of these drugs is the age of the patient population. Skin, like other organ systems ages and as it ages it looses some of its structure and function including its ability to regulate body heat and maintain fluid balance, see Table 3. Usually this is not a problem in the performance of pharmacokinetic trials as it is usually much easier to recruit older subjects than young children. In such situations where sufficient numbers of subjects exist, a secondary pharmacokinetic analysis using both gender and age as covariates should also be undertaken.

In contrast, in the pediatric population, especially in the neonate, differences in skin maturation can be profound in relation to disease severity. In adults the skin represents, on average, only 3% of total body weight while in neonates it can go as high as 13%. This coupled with the fact that the ratio of surface area to body weight in neonates is four times that of adults, suggests that our relationships between surface area and volume need to reconsidered. While term infants are born with and acquire all the characteristics of an intact skin barrier, these high ratios of surface area to weight would only tend to enhance the potential for circulating levels of topically applied drugs to occur after application. In this situation, extrapolation of in vivo biostudy results should be limited to that of younger aged subjects to older subjects and not vice versa.

Because of the increased body weight to surface area ratio in children, the absence of circulating plasma levels in children with the same relative degree

TABLE 3 Functions of Human Skin that Decline with Age

Cell Replacement	Immune Responsiveness
Injury Response	Vascular Responsiveness
Barrier Function	Thermoregulation
Chemical Clearance	Sweat Production
Sensory Perception	Sebum Production
Mechanical Protection	Vitamin D Production

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of surface area involvement, compared to adults, would be supportive of the clinical safety findings across these populations. By the same token, extrapolation of safety from adults to children is not possible for the same reasons. In general, to obtain approval of a pediatric dosing regimen, one has to study the age range in question with adequate numbers of subjects being present at the lowest desired age ranges. While there is no hard and fast rule as to the number of pediatric subjects required, the protocol should prespecify the numbers of children at each age grouping (1–6 months, 6 months-2 years, 2–6 years, etc.). It should also indicate that the enrolled children should be evenly distributed throughout the age range, if not enriched at younger ages, to prevent clustering at the upper ages.

Dosing Instructions

Site preparation prior to the application of a topical product primarily consists of washing the area of application with mild soap and water and patting dry. Care should be taken to avoid the use of harsh soaps and detergent like "liquid soaps" that would tend to strip out the natural oils present in the skin and potentially alter drug delivery. The product should be applied, according to directions, to the affected site, minimizing the exposure or "normal" skin. Following application the subject should follow the specific directions for the product concerning the use of a bandage or occlusive barrier either of which could contribute to enhanced systemic absorption. As a general rule the site should be allowed to air dry naturally following application, before covering the area with clothes.

Special Situations—Minimal Surface Area Application

Across the spectrum of dermatologic conditions there are those conditions like atopic dermatitis that can involve >90% of the total body surface area and those that involve <1% or so of body surface area. Disease states in this latter category include basal cell carcinoma and warts. These lesions are usually circumscribed in nature being distinct from the surrounding skin surface. Treatment of these lesions can include surgical removal and the use of topically applied caustic agents (such as high concentrations of salicylic acid or trichloroacetic acid). In these situations, where the destruction of discrete and limited areas of skin are done, the utility of pharmacokinetic monitoring is of questionable value for a number of reasons:

1. The small surface area involved
2. The destructive nature of the "drug" being applied to the lesion
3. The single use/application nature of these products.

In these situations, even the use of a minimal pharmacokinetic sampling strategy becomes complicated, as one of the precepts of regulatory

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decision-making is not to place the research subjects at any unnecessary risk. While minimal, the act of drawing blood from a patient does carry with it some risk. For these reasons, depending upon the ultimate surface area to be treated at any one time and the total cumulative dose to be applied at any one time, it may be possible to obtain a waiver of in vivo bio-testing. Such considerations should be discussed with the regulatory authorities early on in the development of a topical treatment for these diseases and should not be assumed as a matter of course.

RECTAL DOSAGE FORMS

Since the early 1800s when cocoa-butter suppositories were first developed by the French, use of the rectal route for drug administration has often been proposed as an alternative method to avoid first-pass metabolism and as a viable route of drug delivery in patients who cannot use oral dosage forms. Today suppository dosage forms range from the original cocoa-butter formulations, to those utilizing new polymers and dispersive systems (including the use of oral controlled-release products) designed to overcome one or the other problems associated with rectal administration. Even so, the use of the suppository route in general, and the rectal route in particular is one that is not often pursued in the course of modern drug development. The only exception to this general statement is the proliferation in recent years of antifungal and hormonal products in the form of vaginal suppositories. Rectal suppositories, in comparison, are almost never developed as a first route of administration and rarely as a line extension, except for use in the infant or pediatric population.

Anatomy and Physiology of the Rectum

The rectum is the terminal end of the gastrointestinal (GI) tract. Its primary function, different from any other portion of the GI tract, is not to absorb nutrients or regulate fluid balance but to serve, in much the same way as the bladder, as a holding place for waste materials prior to the regular daily expulsion of these materials. The rectum is muscular in nature and does have a high degree of vascularization, see Fig. 8.

One of the misconceptions regarding rectal drug delivery is that it bypasses first-pass metabolism by avoiding the portal circulation. This is only partially true. The superior, middle, and inferior rectal veins accomplish the removal of blood from the rectal tissues. These veins are interconnected through numerous anastamoses and as such represent a unified drainage system. Of these three veins, the superior rectal vein does drain into the portal vein, thus providing vascular access to the liver. Because of individual variability in the

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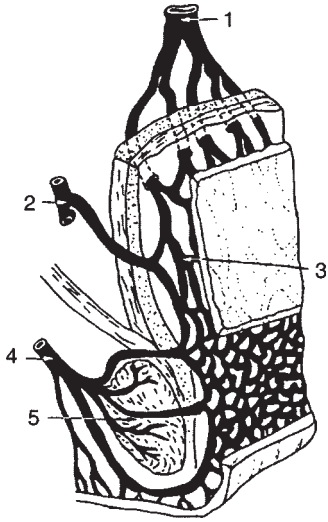

FIGURE 8 (1) Superior rectal vein; (2) middle rectal vein; (3) submucus venous plexus; (4) inferior rectal vein; (5) external rectal sphincter. *Source:* Ref. 49, p. 119.

number and size of the anastamoses present in each individual's venous system the degree of first-pass metabolism in an individual cannot be estimated a priori. Rectal bioavilability should then be expected to be "intermediate" that is lying somewhere between that of an intravenous dose and an oral dose.

No matter if 18th or 21st century technology is used, the primary obstacle to the delivery of drugs from rectal tissue is that these tissues are not inherently permeable to drug absorption. The combination of a relatively small surface area for absorption (~200 cm^2) coupled with the small amount of fluid present and the lack of the specialized structures for absorption (i.e., the villi that line the small intestine) making the rectal environment a poor one for absorption to occur.

Because of these factors the primary mechanism for drug absorption in the rectum, as with the other routes of administration discussed in this chapter is via passive diffusion. Here, however, drug absorption is dependent not as heavily on the permeability of the rectal tissue, but on the amount of drug available in solution ready for absorption. Here the small volume of fluid present in the rectum and the melting/release of the drug from the suppository vehicle can play the major role in retarding drug absorption. In some instances, this can be a desired effect as in the use of controlled-release oral dosage forms of narcotics placed in the rectum for systemic drug delivery and pain relief.

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Drugs for Rectal Administration

For the most part, the rectal administration of drugs is limited to those drugs being used to treat a lower GI condition such as constipation, or when the upper GI tract is compromised either due to disease or for surgical reasons (i.e., patients awaiting surgery). A review of a standard reference such as the RED BOOK or the Physicians Desk Reference reveals very few approved suppository products in the United States. In theory, any drug could be administered via the rectal route, and in some countries the use of suppository dosage forms is relatively popular. The relative lack of approved suppository preparations in the United States is due to a number of factors that are presented in Table 4.

The primary classes of drugs approved as suppositories in the United States are the antinauseants (promethazine, prochlorperazine, etc.) and antipyretics (e.g., acetaminophen). Of the approved agents, acetaminophen, because of its use in the pediatric population, is the most widely used rectal suppository in the United States.

TABLE 4 Some PROS and CONS of Rectal Administration

PROS	CONS
1. Can be used in the unconscious patient or in the patient who cannot swallow.	1. Conditions in the rectum are not favorable for drug dissolution or release (i.e., small volume of fluid present, alkaline pH).
2. Avoids contact between the drug and digestive juices that might otherwise lower bioavailability.	2. Insertion of foreign body into rectum can trigger defecation and subsequent loss of either all or a portion of administered drug.
3. Taste masking of bitter or unpleasant drugs. (Especially important when nausea and vomiting are present.)	3. Patients with constipation may have chronically full rectum due to loss of rectal muscle tone, drug could be adsorbed into fecal matter instead of being absorbed.
4. Can be self-administered.	4. Potential for local microbial drug metabolism of drug prior to absorption.
5. First-pass effect reduced, but *NOT* eliminated.	5. Can be difficult to self-administer in the elderly.
	6. Psychosocial issues and perceptions of this route of administration.

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Bioavailability and Bioequivalence Considerations

General Design Factors

From a bioavailability/bioequivalence point of view, the precepts to be used in designing and executing pharmacokinetic trials with suppository dosage forms are very similar to those surrounding oral dosage forms. Usually rectal suppositories will represent a line extension of an existing product with which safety and efficacy has already been demonstrated. In such cases, the biopharmaceutic program should be concerned not so much with establishing bioequivalence between the dosage forms, an unlikely occurrence, but in demonstrating that therapeutic levels can be achieved within a meaningful therapeutic time window.

As mentioned previously, acetaminophen is the most common rectal suppository in the United States. In Fig. 9, we see the comparison of an acetaminophen containing rectal suppository to an oral dosage form, both dosed at approximately 13mg/kg. As would be expected, the suppository dosage form produces levels which lag behind and below those produced by the oral route. Rectal bioavailability was approximately 78% relative to the oral route, suggesting that a dose of ~16mg/kg would have been required via the rectal route to provide a similar degree of exposure. These results are typical of those associated with rectal dosing and reflect the poor nature of the rectal environment in regard to absorption. It also highlights the fact that dosing ranges determined from oral dosing may not be relevant with regard to rectal administration. Independent dose-ranging trials, guided by the results obtained with oral dosing, should be undertaken to assure that

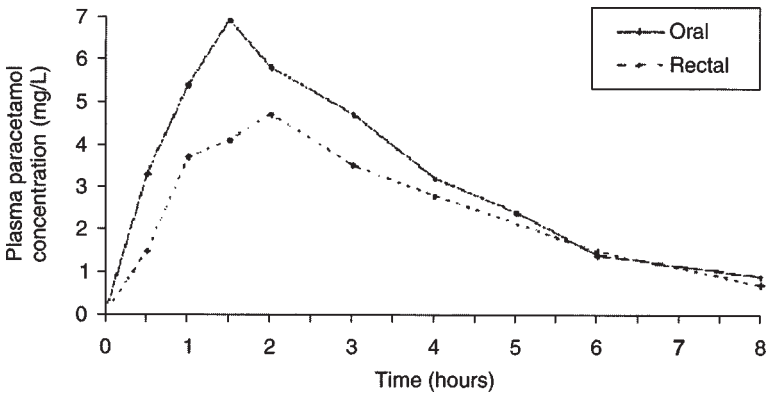

FIGURE 9 Comparison of an acetaminophen containing rectal suppository to an oral dosage form, both dosed at approximately 13mg/kg. *Source:* Ref. 50, p. 427.

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when the rectal route of administration is utilized it results in a safe and efficacious response.

As with oral dosage forms, the in vivo evaluation of suppositories should include an assessment of dosage form proportionality: Specifically, are the release characteristics of a drug from different strength suppositories the same and will these changes have an impact on the clinical utility of the drug. An example of this type of comparison is contained in Table 5 where the results of an in vivo biostudy, using acetaminophen suppositories along with an oral reference dose, are compared for two different strength suppositories. It is clear from this data that the larger 1000 mg suppository actually delivers less drug, albeit for a more prolonged period (note the Tmax difference), than the 2×500 mg suppository treatment. The authors speculate that these differences could be related to the larger total surface area to unit volume/dose for the two-suppository treatment relative to the single suppository treatment. This increased surface area exposes more of the suppository for melting/dissolving, thus increasing both the rate and potentially the bioavailability of the drug substance from the suppository matrix. In either event, an assessment of dosage-form proportionality is essential for the development of proper clinical dosing recommendation for a suppository dosage form.

In vitro Methods

The assessment of in vitro release of drug from suppositories has primarily been limited to the use of melting tests and the use of modified dissolution apparatuses (specifically modified flow-through cells). Such tests, while acceptable from a quality control point of view as a release specification, are insufficient for the assessment of in vivo bioavailability.

Dosing Instructions

Because of its anatomical location subjects should be counseled or the proper use, i.e., insertion, of suppositories. Subjects should be well hydrated, and

TABLE 5 Pharmacokinetic Results Following Oral and Rectal Administration of Acetaminophen to 19 Healthy Adults (mean +/- Std. Dev.)

	Cmax (ug/ml)	Tmax (hr.)	AUC0-24hr. (mg*1/hr)	F*
Tablet 2 × 500 mg	19.1 +/- 1.7	0.6 +/- 0.1	55.7 +/- 5.3	—
Suppository 2 × 500 mg	6.9 +/- 0.7	2.4 +/- 0.4	43.1 +/- 4.9	77.3
Suppository 1 × 1000 mg	3.6 +/- 0.5	6.4 +/- 0.7	36.6 +/- 4.9	65.7

*Relative to oral dosing. *Source:* Ref. 54.

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should have had a bowel movement at least an hour prior to insertion to minimize both the loss of drug to rectal contents (adsorption) and the potential to trigger a bowel movement by inserting the suppository. The subject should be instructed to lie on their left side with the left leg straight and the right leg bent up towards the chest. In adults, the suppository should be inserted 2–3 inches into the rectum with lesser insertion distances being used in children depending upon their age. After insertion and retention of the suppository, the subject should be instructed to remain in this position for at least 20min prior to engaging in other activities.

CONCLUSIONS

As has been shown in this chapter, the development of alternative routes of drug delivery require careful consideration of the disease state to be treated, the physiochemistry of the formulation, and the site and manner of drug application/delivery. Although, physically, widely separated, the intranasal, topical, and rectal routes of administration share certain similarities in that the tissues associated with these routes are not normally thought of as sites of drug absorption. Because of this, drug development for these alternative routes requires a thorough knowledge of both the disease state being treated with regard to effective plasma levels and the time course of their attainment. It is because of the limitations that these routes of administration place on absorption that one often needs a separate dosing strategy to ensure efficacy consistent with oral dosing. In vitro methodologies, while useful in lessening the regulatory burden with the oral route of administration, are less applicable here due to both methodological short-comings and the lack of a demonstrated correlation with in vivo events.

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Scientific and Regulatory Issues in Development of Chiral Drugs

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BACKGROUND

General principles of drug development are to conduct experiments and clinical studies which provide the information necessary to assess drug's safety, efficacy, and dosage adjustments to make in specific population. Having chirality in the drug molecule being developed adds additional challenges which should be resolved. This chapter will provide a brief introduction to chirality and its implications on pharmacokinetics and

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pharmacodynamics. Further, regulatory considerations for chiral drugs will also be discussed.

TERMINOLOGY

Chiral vs. Achiral

Chirality is a geometric attribute; a molecule or object which is not identical to (or nonsuperimposable upon) its mirror image molecule or object is said to be chiral. By the same criteria, a molecule or object is said to be achiral if it is identical to (or superimposable upon) its mirror image molecule or object. More simple definition for chiral molecule can be stated as "a molecule that contains one or more asymmetric centers within its molecular structure" or "molecules that have at least a pair of enantiomers."

Stereoisomers

Stereoisomers can be defined as molecules consisting of the same chemical constituents (or groups) with the same structural formulas but differ only with respect to the spatial arrangement of certain atoms or group of atoms [1]. They can be subclassified into: (a) *optical isomers* and (b) *geometrical isomers*. Optical isomers are a set of Stereoisomers, at least two of which are optically active or chiral. Geometric isomers, on the other hand, are members of set Stereoisomers that contain no optically active centers.

Enantiomers

Two Stereoisomers in which molecules are nonsuperimposable mirror images of one another are said to be enantiomers. Enantiomers differ only in the spatial arrangement of ligands attached to the chiral center, but they share the same physicochemical properties such as refractive indices, melting points, boiling points, and solubility. Enantiomers are sometimes referred to as optical antipodes, where *anti* means opposite *and podes* means feet.

Diastereoisomers

Stereoisomers with two or more asymmetric centers and whose molecules are not mirror images of one another are said to be diastereoisomers, or simply diastereomers. Unlike enantiomers, diastereomers can differ in physicochemical properties such as signs and magnitudes of optical rotations, melting points, solubilities, and refractive indices. The most common diastereomeric molecule is one that contains two asymmetric

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carbons. This situation is illustrated by the compounds ephedrine and pseudoephedrine. Each diastereomer of ephedrine and pseudoephedrine exists as a member of an enantiomeric pair, i.e., d- and 1-ephedrine and d- and 1-pseudoephedrine, respectively. Thus, diastereomeric molecules with two asymmetric centers are most often represented by four stereoisomers. Diastereoisomers and geometric isomers are both chemically distinct and pharmacologically different. They are generally readily separated without chiral techniques.

Racemic Mixture

An equal (1:1) mixture of two enantiomers is said to be racemic. The IUPAC rules [2, 3] state that "when equal amounts of enantiomeric molecules are present together, the product is termed racemic independently of whether it is crystalline, liquid or gaseous." Thus in the IUPAC rules the word "racemic" (adjective) is applied to an optically inactive product in any state of matter, and "racemic mixture" would appear to be the correct terminology for a 1:1 mixture of enantiomers in any physical state. A racemic mixture, therefore, is a 50:50 mixture of the two enantiomers of a chiral compound. Conversion of one enantiomer to a 1:1 mixture of the two is referred to as racemization. Because the two enantiomers have equal and opposite specific rotations, a racemic mixture has a specific rotation of zero, i.e., it is optically inactive. In nature, most naturally occurring compounds occur as a single enantiomer, not as racemic mixtures. The importance of racemic mixtures is that ordinary laboratory synthesis which generates a stereogenic center produces a racemic mixture.

Optical Activity

A physical property that distinguishes two enantiomers is "optical activity," which refers to the property of chiral compounds of rotating the plane of plane-polarized light to the right (clockwise) or to the left (counter-clockwise). The two enantiomers have exactly the same ability to rotate the plane of monochromatic plane-polarized light, quantitatively, but they rotate it in opposite directions. Thus, if one enantiomer rotates the plane by 10 degrees clockwise (considered a positive rotation), the other rotates it by -10 degrees in the counterclockwise direction (considered a negative rotation). Since the exact amount of the rotation of the plane by a given enantiomer depends upon how much of that enantiomer the light encounters as it passes through the solution, the measured rotation is divided by the concentration of the enantiomer and by the path length of the polarimeter cell to give a true measure of the inherent ability of the enantiomer to rotate the plane of polarized light. A positive rotation is also

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referred to as dextrorotation and a negative rotation is called levorotation, and denoted by d and 1 respectively, and the terms dextrorotatory and levorotatory are superseded by (+) and (-), respectively [1].

NOMENCLATURE OF STEREOISOMERS

The Fischer Convention [4]

The configuration of an asymmetric center was initially determined by the chemical transformation of the chiral molecule to an arbitrarily selected standard, (+)-glyceraldehyde. This was the basis of the Fischer Convention for the determination and designation of configuration. The system operates by relating the configuration at the asymmetric center of the molecule under investigation to (+)-glyceraldehyde, which was arbitrarily assigned the D configuration. To assign a configuration, the molecule under investigation must be chemically converted to glyceraldehyde or to another molecule of known configuration. After this is accomplished, the sign of rotation is determined and the D or L configuration is assigned accordingly. The sign of rotation cannot be employed prior to assigning a configuration, because they do not always correspond. For example, L-alanine has a (+) sign of rotation, whereas the sign of rotation for L-glyceraldehyde is (-). The Fischer Convention is widely used to assign a configuration for sugars, which contain a number of asymmetric centers. For diastereomers with only two centers, the Fischer Convention assigns a series as D or L according to whether the configuration at the highest numbered asymmetric center is analogous to D- or L-glyceraldehyde. The Fischer Convention is often incorrect and difficult to use, especially when complex chemical transformations are required to convert the molecule under investigation into a molecule of known configuration. In addition, the assigned configuration, D or L is often confused with the observed sign of rotation, d or 1. Because of the potential confusion that it could lead, the Fischer Convention has been almost entirely replaced by the Cahn-Ingold-Prelog Convention.

The Cahn-Ingold-Prelog Convention [5]

The Cahn-Ingold-Prelog Convention was designated by its originators as the "sequence rule," since it designates the sequence of substituents around the asymmetric center. In this method, the substituents at the chiral center are first sized according to their atomic number from the largest to the smallest. Once the rank order is determined, the molecule is held so that the lowest group in the sequence is pointed away from the observer. Then if the other groups listed in the descending order of precedence are oriented

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clockwise, the molecule is designated *R* (rectus), and if counterclockwise, *S* (sinister). In the example presented in Fig. 1, the order is L (large), M (medium), S (small), and S' (smallest). The molecule is then oriented so that the smallest (S') substituent is directed away from the observer. The configuration is then determined by whether the sequence L-M-S goes in a clockwise or counterclockwise direction. A clockwise direction is designated as *R (rectus)* whereas the counterclockwise direction is designated as *S (sinister)*. This convention can be used to rapidly and unambiguously specify the configuration of a chiral center. If one enantiomer has an *R* designation, its antipode or mirror image has the *S* configuration. The Cahn-IngoldPrelog Convention (see Fig. 1) is also very useful for describing diastereomers. In the case of diastereomers, each chiral center is designated independently and the configuration of the whole molecule can be conveniently assigned. For example, instead of d- and 1-pseudoephedrine, the assigned configurations are *(R, S)*- and *(S, R)* ephedrine and *(R, R)*- and (S,S)-pseudoephedrine. The enantiomeric relationships within the ephedrine and pseudoephedrine molecules and the diastereomeric relationship between ephedrine and pseudoephedrine are recognized clearly.

A set of terms are also in use to describe the pharmacological activity of stereoisomers. In an enantiomeric pair, the isomer with the greater pharmacological affinity or activity is known as *eutomer,* and the one with the lower pharmacological affinity or activity is called *distomer* [6]. The ratio of affinities or activities of eutomer to distomer is referred to as the *eudismic ratio,* and the logarithm of eudismic ratio is known as *eudismic index*. Slope of a plot of eudismic index vs. the logarithm of affinity of eutomer (ideally expressed either pA_2 or pD_2 values in pharmacology, or K_i or K_m values in enzymology) for a homologous series is called the *Eudismic Affinity Quotient (EAQ)*. It represents a quantitative measure of the stereoselectivity within compound series for a specific biological effect [7, 8]. The greater the difference in pharmacological activity between a pair of enantiomers, the greater will be the specificity exhibited by eutomer, and this is referred to as Pfeiffer's Rule. A positive slope of EAQ reflects such

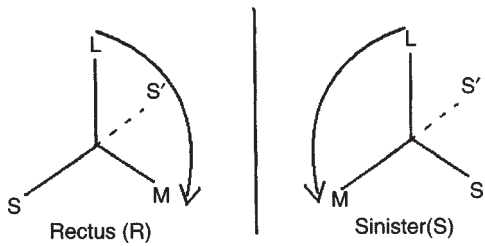

FIGURE 1 Cahn-Ingold-Prelog Convention.

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greater difference. However, it should be noted that exceptions to Pfeiffer rule have been reported [9].

STEREOSELECTIVITY

Stereoselectivity (or enantioselectivity) in pharmacology as well as pharmacokinetics following administration of racemic drug has been recognized since early part of last century. In the past few decades, pharmacological and pharmacokinetic investigations have clearly demonstrated significant differences in the biological activity of some isomeric pairs. Following is a concise review of Stereoselectivity with regard to pharmacodynamics and pharmacokinetics of racemic drugs.

Pharmacodynamic Considerations

From the pharmacodynamic and therapeutic standpoint, multiple outcomes are possible with racemic drugs. Following is a brief discussion on three categories of racemic drugs based on the qualitative and quantitative activities of stereoisomers. It should be noted that many drugs may belong to more than one category, and with ever-growing knowledge of stereochemistry of drug action and disposition, they may be more appropriately placed into the relevant category.

Racemates in which one Stereoisomer Possesses the Majority or all of the Beneficial Activities and the Other Isomer is Inactive

It is less common, although highly desirable, to have all the activity in one enantiomer. This necessitates development of single isomer, avoiding the unwanted activity/toxicity of the antipode. Selected examples where one member of an enantiomer pair was pharmacologically active and the other inactive include α-methyldopa (antihypertensive activity) [10, 11] and propranolol (β-blocking activity) [12]. In the case of beta-blockers representing the aryloxyproponolamine category, the therapeutic effect resides almost entirely in the *S*-stereoisomer. For example, the eudismic ratios of three beta-blockers, atenolol, propranolol, and metoprolol, are 12, 130, and 270, respectively. The inactive (or less active) enantiomers of these beta-blockers are not known to cause any serious side effects.

- *Racemates in which both enantiomers have similar potency*

Although it is quite common for enantiomers to possess similar qualitative pharmacological activity, it is uncommon that both isomers possess similar qualitative and quantitative activity profiles. Examples where similar qualitative activity was observed for many enantiomeric pairs, some of which include promethazine (with respect to antihistaminic activity) [13],

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flecainide (with respect to electrophysiological effects) [14], warfarin (anticoagulant activity) [15, 16], and verapamil (vasodilator effects) [17, 18]. In such cases with racemic drugs, the separation of two enantiomers may not be justified. However, although the two enantiomers may be qualitatively and quantitatively similar with respect to the main therapeutic activities for which they are indicated, subtle differences with regard to other activities are possible which must be carefully addressed.

■ *Both enantiomers qualitatively and quantitatively differ in their activity*
Stereoisomers may sometimes exhibit desirable, but different biological effects such that both may be marketed with different therapeutic indications. For example (8S, 9R), quinine is an effective antimicrobial agent, while the corresponding (8R, 9S) diastereomer quinidine is an antiarrhythmic agent. Other examples of enantiomers that have completely different (qualitative) activities include propoxyphene (the d-isomer has analgesic activity and the 1-isomer exhibits the antitussive properties) and sotalol (where the d-isomer is a type 3 antiarrhythmic while 1-sotalol is a ß-blocker). The two optically active isomers of indacrione have qualitatively and quantitatively different diuretic and uricosuric activities [19]. Sometimes Stereoisomers possessing different pharmacodynamic activities may be developed as racemates because the combination offers a therapeutic advantage. For example, (*R*)-enantiomer of indacrinone has a diuretic activity and causes uric acid retention, whereas the *S*-enantiomer possesses uricosuric activity and promotes the secretion of uric acid. This combination may be beneficial to induce diuresis in hypertensive patients who typically have elevated uric acid levels.

Pharmacokinetic Considerations

Absorption

Drugs, in general, are absorbed by passive diffusion, a process dependent upon physicochemical properties of diffusant molecule such as aqueous/lipid solubility, ionization, and molecular size. Since enantiomers do not exhibit differences in their physicochemical properties, stereoselectivity is not expected. However, diastereoisomers may exhibit differences in their absorption profies as they differ in their physicochemical properties. Drugs that are transported via carrier-mediated mechanisms (e.g., facilitated diffusion or active transport processes) may exhibit significant stereoselectivity. This is because the process of carrier-mediated transport involves a specific interaction of the drug with a chiral endogenous macromolecule. For example, it has been reported that L-isomer is preferentially absorbed compared to the *D*-enantiomers for dopa and methotrexate [20, 21]. The transport systems involving P-glycoprotein-mediated efflux mechanisms are

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also potentially stereoselective. Interestingly, some stereoisomers have been shown to facilitate the absorption of their optical antipodes. For example, the bioavailability of *S*-propranolol is greater when administered as a racemate than as a single isomer, suggesting that *R*-propranolol promotes the absorption of *S*-isomer [22].

Distribution

As with drug absorption, distribution of drugs is generally described by passive diffusion. Stereoselectivity in drug distribution may occur as a result of binding of drugs to either plasma or tissue proteins and/or transport via specific tissue uptake and storage mechanisms. Difference between enantiomers in plasma protein binding have been reported for a number of drugs. A majority of drugs bind in a reversible manner to plasma proteins, notably to human serum albumin (HSA) and/or alpha1-acid glycoprotein (AGP). Acidic drugs bind preferentially to HSA, with binding at site II (benzodiazepine site) on the protein generally displaying greater enantiomeric differences than at site I (warfarin site) and basic drugs predominately bind to AGP. It should be noted that Stereoselectivity in binding may vary for different proteins, e.g., the protein binding of propranolol to AGP is stereoselective for the *S*-enantiomer, whereas binding to HSA favors (*R*)-propranolol [23]. In whole plasma the binding to AGP is dominant such that the free fraction of the *R*-enantiomer is greater than that of (*S*)-propranolol.

Enantioselective tissue uptake, which is in part a consequence of enantioselective plasma protein binding, has been reported. For example, the uptake of ibuprofen into lipids is stereoselective in favor of the *R*-enantiomer, but this is as a result of stereospecific formation of the acyl-CoA thioester followed by incorporation as hybrid triglycerides [24].

Metabolism

Drug metabolism, involving phase I as well as phase II biotransformations, shows Stereoselectivity. Enantioselectivity in drug metabolism may be described as the rule rather than the exception and probably is responsible for the majority of the differences observed in enantioselective drug disposition. Stereoselectivity in metabolism may arise due to differences in the binding of enantiomeric substrates to the enzyme active site and/or be associated with catalysis due to differential reactivity and orientation of the target groups to the catalytic site. As a result, a pair of enantiomers are frequently metabolized at different rates and/or via different routes to yield alternative products. Examples include propranolol, verapamil, and warfarin. For example, *S*-isomer of propranolol is metabolized predominantly

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by glucoronidation, whereas R-isomer undergoes oxidative degradation to form 4-hydroxypropranolol. Enantioselectivity in metabolic clearance is more apparent for drug molecules undergoing first-pass enterohepatic metabolism.

The stereoselectivity of drug metabolic processes may be classified into three categories in terms of their selectivity with respect to the substrate, the product or both. An alternative classification involves the stereochemical consequences of the transformation reaction, and according to this approach, metabolic pathways may be divided into five groups: (a) prochiral to chiral transformations, (b) chiral to chiral transformations, (c) chiral to diastereoisomer transformations, (d) chiral to achiral transformations, and (e) chiral inversion.

Chiral Inversion. The process of metabolic conversion of one stereoisomer into its enantiomer with no other alteration in structure is known as chiral inversion. Examples of agents undergoing this type of transformation are the 2-arylpropionic acid (2-APAs) nonsteroidal antiinflammatory drugs (NSAIDs) such as ibuprofen, fenoprofen, flurbiprofen, ketoprofen [25] and the related 2-aryloxypropionic acid herbicides, e.g., haloxyfop [26]. In the case of the 2-APAs the reaction is essentially stereospecific with the less active, or inactive, *R*-enantiomers undergoing inversion to the active *S*-enantiomers. Following administration of *(S)*-stiripentol the *R*-enantiomer produced by racemization undergoes conjugation with glucuronic acid and excretion in the bile, the *S*-enantiomer appearing in the systemic circulation, whereas following administration of *(R)*-stiripentol the glucuronidation pathway is saturated and both enantiomers, *(S)*-stiripentol being formed in the gastric acid, are found in the systemic circulation [27]. For drugs exhibiting chiral inversion, the residence time of the drug in the gastrointestinal tract affects the eudismic ratio. As an example, the relative concentration of the pharmacologically active *S*-enatiomer of ibuprofen (*S:R* ratio) increases with prolongation of the GI transit time of racemic formulations due to a corresponding increase in chiral inversion of the *R*- to *S*-enantiomer in the gut. In such cases, administration of *S*-ibuprofen and not the racemate, therefore, reduces the formulation-dependant variability in the concentration of the active enantiomer in the body.

Renal Clearance

Stereoselectivity in renal excretion may occur with all aspects of renal clearance including protein binding, glomerular filtration and passive reabsorption, or active secretion or reabsorption. Enantioselectivity in renal clearance has been reported for a number of drugs and in many cases the selectivity is relatively modest with enantiomeric ratios between 1.0 and

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3.0. The diastereoisomers quinine and quinidine show enantioselectivity in renal clearance where the difference is about four fold with values of 24.7 and 99 mL min^{-1} in man, respectively [28]. In another case, concurrent administration of probenecid has been shown to stereoselectively reduce the renal clearance of (-)-isomer of sultopride, but not that of the (+)-enantiomer following administration of the racemic drug to rats [29]. In contrast, coadministration of the racemic drug with procainamide lead to significant reductions in both total and renal clearance of both the enantiomers [29]. Stereoselective renal clearance may also occur for metabolites. For example following the repeated oral administration of the individual enantiomers of disopyramide, significant differences in both the total and unbound renal clearances of the monodesisopropyl metabolite were observed, both processes being Stereoselective for the (+)-*S*-enantiomer [30]. In contrast the total renal clearance for the drug showed no stereoselectivity, whereas the unbound renal clearance of *(S)*-disopyramide was greater than that of the *R*-enantiomer. The renal elimination of both enantiomers of both the compounds was associated with tubular secretion and the possibility exists that drug-metabolite-enantiomer interactions in renal tubular secretion may occur [30]. Stereoselective elimination may greatly influence pharmacodynamic parameters, including intensity and duration of action for drugs eliminated primarily by renal clearance. From a clinical standpoint, a less potent, but slowly cleared isomer offers greater advantage than a highly potent, rapidly cleared enantiomer.

Protein Binding

Enantiomers of many chiral drugs have shown differential affinities toward human plasma proteins. Much of the drug, in general, bind to different extents to one or more of the different blood elements such as cells and proteins when reach the systemic circulation. Protein binding of some enantiomers to plasma proteins, albumin, and $alpha_1$-acid glycoprotein (AAG) may be Stereoselective. The high affinity binding sites on albumin have more receptor-like properties than the binding sites on α_1-acid glycoprotein, since the former can more effectively differentiate between different drug enantiomers than the latter. Acidic drugs, such as warfarin and active metabolites of diazepam and oxazepam, bind stereoselectively to serum albumin, whereas basic drugs such as verapamil and disopyramide bind stereoselectively to AAG [31–33]. For drugs exhibiting enantioselective protein binding, one should carefully evaluate the dynamics of the racemic mixture to determine the concentration of the free, unbound drug at the target site to assess its clinical activity and toxicity.

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REGULATORY CONSIDERATIONS

Despite the challenges identified with some racemates, the common practice of developing drug products of racemates has led to an ongoing discussion on the rationale and the regulatory aspects of chiral drug product development by the scientific community [34–39]. This section presents a discussion on regulatory issues relating to the pharmaceutical development of stereoisomers, particularly those with one or more chiral centers. Guidelines for development of chiral drugs have been issued by European, Candian, United States, and other regulatory agencies [40–42]. Some of the guidance documents are (1) FDA's policy statement for the development of new stereoisomeric drugs, issued by the FDA in 1992. (2) Bioavailability and Bioequivalence Studies for Orally Administered Drug Products—General Considerations (March 2003). (3) Investigations of chiral active substances issued by commission of the European countries in 1994. (4) Stereochemical issues in chiral drug development, issued by Therapeutic Product Programme, Canada (2000).

As discussed earlier, stereoisomers are often readily distinguished by biological systems and may exhibit different pharmacokinetic properties including absorption, distribution, metabolism, and excretion. Consequently, quantitative and/or qualitative differences in pharmacologic and/or toxicologic effects are possible with racemic drugs. When stereoisomers are biologically distinguishable, they may behave as different drugs. Regardless of this behavior, it has been past practice to develop chiral drug products as racemates. There are many reasons for such a practice. Some of the products that are racemates were marketed at a time when good separation and/or synthetic procedures for individual enantiomers were not available for manufacture on a commercial scale. Some of these products date to before 1938 when extensive new drug applications (NDAs) were not required for marketing of a new drug. In some cases, enantiomers were found to be identical in pharmacological properties. In other cases, one enantiomer was inert or possessed little or no biological activity. Since commercial separation of racemates was less common, the question of developing individual enantiomers as drug products was largely of academic interest. The technological advances over the past 25 years, including largescale chiral separation procedures or asymmetric synthesis, make it possible to produce many single enantiomers on a commercial scale. Consequently, the need for the regulatory policies and guidelines with respect to the development of stereoisomeric mixtures has grown over the years. It follows that the development of chiral drugs presents a number of issues, each of which is recognized as an important consideration [40]. These may include:

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- acceptable manufacturing control of synthesis and impurities
- acceptable enantiomeric assays
- adequate pharmacologic and toxicologic assessment
- proper characterization of metabolism and distribution
- assessment of chiral inversion
- appropriate clinical evaluation

Among the stereoisomers, geometric isomers and diastereomers should be treated as separate drugs and developed accordingly. However, with the rare exception of cases where in vivo interconversion occurs, the development of mixtures of geometric isomers or diastereomers is generally not justified unless they, by chance, represent a reasonable fixed dose combination [41]. In such cases, whether the optimal ratio of the two isomers is the ratio produced by an unmodified synthesis should be carefully examined. Geometric isomers, in general, have been developed as single isomers, whereas practice with respect to diastereomers has been variable.

Since most biochemical processes are stereospecific, chiral substances from natural sources are normally obtained in an optically active form. For example, antibiotic products prepared by fermentation are mostly stereospecific. Products prepared by different biochemical processes, however, may have different configurations. Lactic acid produced by fermentation of sugars is levorotatory, while lactic acid produced in living muscle is dextrorotatory [42].

All of the reported synthetic procedures of steroids at one time yielded racemic mixtures. Asymmetric processes were developed for many single enantiomers, some of which employed yeast. Some pharmaceutical firms used both microbiological fermentation and chemical transformations to produce the specific enantiomer. Both of these forms are still on the market [42]. A completely synthesized product, the antihypertensive drug methyldopa is prepared completely as the levo form, since all of the activity lies in it and not with the other enantiomer.

Racemates vs. Enantiomers

Pharmacological assessment of chiral substances in an early research phase can facilitate the selection of either single enantiomer or racemate for drug product development. The pharmacological investigations of enantiomers may reveal different scenarios, some of which are discussed earlier. While inactivity of one member of an enantiomeric pair might be considered trivial and often overlooked, there are instances in which toxicity has been associated with one member of a pair of stereoisomers, not necessarily the active isomer (eutomer). For example, vomiting is caused by the d-isomer of levamisole and myasthenia gravis symptoms have disappeared when the d-

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isomer was removed from d, 1-carnitine. In case of ketamine, the *S*(+)-isomer is three to four times more potent than *R*(-)-enantiomer with respect to the desirable anesthetic and analgesic activities, but the notorious side effects were overwhelmingly linked to *R*(-)-isomer. Further, there are many cases in which enantiomers have exhibited pharmacokinetic differ-ences, the discussion of which is beyond the scope of this chapter. While pharmacological studies have presented us with many different situations for racemates, it is incorrect to expect that the concentration of enantiomers in plasma remain 1:1. Further, it is unreasonable to assume that the optimum ratio of the enantiomeric pair to be the 1:1 ratio of the racemate.

General Policy and Application Submissions for Chiral Drugs

When stereoisomers are considered for drug product development, the sponsor must first decide as to whether to separate the isomers (or synthesize them individually) or to deal with the substance as a racemate for all drug development investigations. These decisions must take into consideration the number of isomers present, the difficulty of separation (or synthesis as the case may be), and the toxicity/effectiveness of the substance. Other considerations in the selection of a particular form of stereoisomers include the route of administration, rates of absorption, mechanism of action, biotransformation, elimination, and biological activity of the isomers.

The stereoisomeric composition of a drug with a chiral center and the quantitative isomeric composition of the material used in pharmacologic, toxicologic, and clinical studies should be known. The final product specifications should assure identity, strength, quality, and purity from a stereochemical point of view.

In order to evaluate the pharmacokinetics (i.e., kinetics of absorption, distribution, metabolism, and excretion) of a single enantiomer or mixture of enantiomers, it is important that one should develop quantitative assays for individual enantiomers in in vivo samples early in drug development. Any potential interconversions between the enantiomers should be carefully evaluated. Failure to take interconversion into account while developing a single enantiomer can result in drug development failure. For example, a racemate which was approved and had efficacy residing in one isomer was being developed as an enantiomer. The sponsor initiated developing active enantiomer using 50% of the dose that was approved as an racemate. However, the fact that, in vivo, about 20% of inactive enantiomer converts to active enantiomer was ignored. Thus, while developing active isomer, 60% of the racemate dose should have been used (to compensate for the interconversion). Since interconversion was not taken into account, it

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resulted in an inconclusive trial. Thus, it is important to take interconversion into account. In general, when the pharmacokinetic parameters of isomers of the racemate drug product are different, manufacturers should monitor the enantiomers individually to determine properties such as dose linearity, the effects of altered metabolic or excretory function, and drugdrug interactions. If the pharmacokinetic profiles for both stereoisomers are found to be identical or a fixed ratio between the plasma levels of enantiomers, an achiral assay or an assay that monitors one of the isomers should be adequate for subsequent evaluation.

If and when possible, the main pharmacologic activities of the stereoisomers should be compared in in vitro systems, in animals, and/or in humans. A relatively mild toxicologic profile of a chiral chemical using the racemate would, in general, support further development without separate toxicologic evaluation of the individual enantiomers. However, if there is any toxicity beyond the natural extensions of the pharmacologic effects of the drug, toxicologic evaluation of the individual enantiomers should be undertaken [40].

While the decision of whether to market a specific isomer or a racemate is one that is primarily under the control of the pharmaceutical firm (or sponsor), it is generally based on the pharmacologic, therapeutic, and toxicological considerations of the intact racemate, individual isomers, stability of the drug, technical feasibility of manufacturing the individual isomer on a commercial scale, and cost of manufacture of individual isomers. Enantioselectivity in pharmacokinetics and/or pharmacodynamics presents four possible combinations of scenarios, that is, neither PK nor PD are enantioselective; only PK or PD are enantiospecific; or PK and PD are enantioselective. When PD (safety and efficacy) of a racemate is enantioselective, one needs to consider if developing an enantiomer is a better option.

When a sponsor submits an IND for either the racemate or the individual isomer, it would be very helpful to the reviewers in the regulatory agencies to have a discussion on why a particular form was chosen to be included in the submission. In some cases, studies of individual isomers have been undertaken as an after-the-fact decision when clinical findings have shown a serious adverse reaction together with an effective response in a particular disease or condition. Early testing of the individual isomers on their pharmacological and toxicological properties would provide informa-tion, which would help the sponsor make a decision on how to proceed with the product development. Should the decision be to develop the racemate, adequate controls and tests must be used to assure that the drug used in animal testing and human trials is identical to that proposed for marketing, and that it can be reproduced in every batch manufactured. Subsequent to the IND submission, FDA invites discussion with sponsors concerning

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TABLE 1 Required Information for Chiral Drug Submissions: Chemistry, Manufacturing, and Controls

When dealing with chiral drugs, the chemistry section should include information to assure the identity, quality, purity, and strength of the drug substance and drug product. In addition, the following considerations should be taken into account:

Methods and Specifications

Drug Substance

Include a stereochemically specific identity test and/or
Include a stereochemically selective assay method

Drug Product

Include a stereochemically specific identity test and/or
Include a stereochemically selective assay method

Stability

Provide method(s) to assess the stereochemical integrity of the drug substance and drug product (if the stereochemical conversion does not occur, stereoselective tests might not be required)

Labeling

Provide a unique established name and a chemical name with the appropriate stereochemical descriptors

Pharmacology/Toxicology

Pharmacodynamics

Characterize activity of the individual enantiomers for the principal pharmacologic effect and any other important pharmacological effects affecting potency, specificity, and maximum effect

Pharmacokinetic Profile

Characterize the pharmacokinetic profile of each isomer in animals and later compared to the clinical pharmacokinetic profile obtained in Phase 1.

Toxicology

It is generally sufficient to carry out toxicity studies on the racemate. However, in the event of unexpected toxicity, the findings should be repeated with the individual isomers to ascertain whether only one enantiomer was responsible for the toxicity.

Impurity Limits

Determine the concentration of each isomer and define limits for all isomeric components, impurities, and contaminants on the compound tested preclinically that is intended for use in clinical trials

Source: Ref. 40.

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whether to pursue development of the racemate or the individual enantiomer and general drug development plan.

The information that is presented in the IND submission must detail the full composition of the drug substance, and include adequate information on the method of manufacture, the starting materials, intermediates, reagents, solvents, catalysts, in-process controls, and final controls. It is imperative that the information on whether the drug is a specific enantiomer, a racemate, or a mixture should be provided. The data submitted on substances that exist as stereoisomers should include a discussion on the possible isomers that may result from the method of manufacture, and the results of studies carried out to investigate the physical, chemical, and biological properties of these isomers. Since enantiomeric differences are common between the different animal species and between animals and humans, as evidenced by the permeation of propranolol enantiomers, it should- be clearly mentioned as to what form was used in the animal studies and what form(s) will be used in the initial use in humans [40]. As stated earlier, drugs in which one of the isomers in a racemate is "inactive" with respect to safety and adverse events, an isomer may be developed for marketing, provided the separation or asymmetric synthesis is economically prohibitive or technically difficult on the large scale. The information that should be generally provided by the sponsor in the drug application submissions, in part, is presented in Table 1.

Development of a Single Stereoisomer After Studies on Racemate

When developing a single Stereoisomer from a racemic mixture that has already been studied nonclinically, appropriate pharmacologic/toxicologic evaluation should be carried out to permit the existing information generated on the racemate to be applied to the pure enantiomer. Continuation of investigations usually include the repeat-dose toxicity evaluation carried out up to three months and the reproductive toxicity in the most sensitive species, using the single enantiomer. A positive control group consisting of the racemate should be included in these studies. If the toxicological profiles of the single enantiomer product and the racemate are similar, no further studies would generally be required. However, if the single enantiomer is found to be more toxic, further investigation should be conducted to offer explanation for that finding and the implications for human dosing should be considered [40].

If the pharmacodynamic and pharmacokinetic differences between the enantiomers are insignificant, racemates may be considered for development. However, development of a single enantiomer may be desirable in some cases where, for example, significant differences in toxic or undesirable pharmacologic effects are seen. The pharmacological and

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toxicological profiles of the individual enantiomers and their active metabolites should be further investigated if the toxicity observed with the racemate at clinical doses is not anticipated from the pharmacology of the drug.

It is important that both the enantiomers should be evaluated clinically and based on these findings, one should consider a racemate or individual enantiomer. When both the enantiomers are pharmacologically active but differ significantly in potency, specificity, or maximum effect, only one isomer should be considered for development. When both the enantiomers exhibit desirable but different properties, development of a mixture of the two, not necessarily the racemate (componds with 1:1 ratio of enantiomers), as a fixed combination might be reasonable [40].

If a racemate is considered for development, the pharmacokinetics of the two enantiomers should be investigated in Phase 1 studies. Any potential interconversion should also be studied. Based on Phase 1 or 2 pharmacokinetic data, it would be possible to determine whether an achiral assay or monitoring of just one enantiomer where a fixed ratio is confirmed will be sufficient for pharmacokinetic evaluation. If a racemate has been marketed and the sponsor desires to develop the single enantiomer, evaluation should include determination of whether there is significant conversion to the other isomer, and whether the pharmacokinetics of the single isomer are the same as they were for that isomer as part of the racemate [40].

Use of Enantiospecific Assays for Assessing Bioavailabilty and Bioeqivalence

Use of enantiospecific assays to assess bioavailabilty and bioequivalence has received considerable attention in the literature. Guidance published by the FDA "Bioavailability and Bioequivalence Studies for Orally Administered Drug Products—General Considerations" addresses this issue and is summarized in Fig. 2. Regulatory guidances on chiral drug development issued by other countries have also addressed the issue of using enantiospecific assay.

In general, for bioavailability studies, measurement of individual enantiomers may be important. For bioequivalence studies, the FDA guidance recommends measurement of the racemate using an achiral assay. Measurement of the individual enantiomers in bioequivalence studies is recommended only when all of the following conditions are met (Fig. 2): (1) the enantiomers exhibit different pharmacodynamic characteristics; (2) the enantiomers exhibit different pharmacokinetic characteristics; (3) primary efficacy/safety activity resides with the minor enantiomer; and (4) nonlinear

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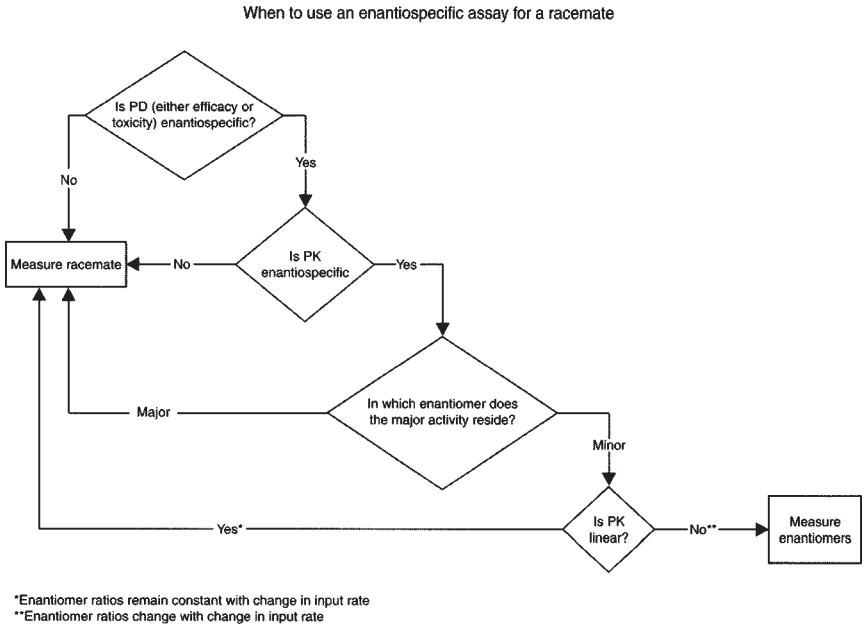

FIGURE 2 Decision tree for use of stereospecific assay for BE studies.

absorption is present (as expressed by a change in the enantiomer concentration ratio with change in the input rate of the drug) for at least one of the enantiomers.

Guidance issued by Therapeutic Products Program (Canada) states that in general, when comparing solid dosage forms of similar type (e.g., two immediate release formulations), total drug concentrations can be measured. Bioequivalence comparisons should be made between "pharmaceutically equivalent products." The bioavailability of each enantiomer should be compared in the following cases:

a. bioequivalence studies for comparison of different types of solid oral dosage forms, e.g., comparison of a modified release drug product to an immediate-release product, or to a different kind of modified-release formulation.
b. If the in vivo enantiomeric ratio is affected due to differences in release rates or absorption of the drug substance, or if the drug shows enantioselective nonlinear first-pass metabolism.

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS: CASE STUDIES

Presently, there are several racemates and individual enantiomers of previously approved racemates being marketed. Some of the examples include citalopram, which is a racemate, escitaloprarn (the *S*-isomer of citalopram), esomeprazole (*S*-isomer of omeprazole), and focalin (the dextrorotary isomer of methylphenidate), which are the single isomers of already-approved racemates, etc.

Due to space limitations in this book, we cannot get into details of what kind of studies were submitted for approval of these products. However, readers can learn a great deal about the regulatory submission for any drug by refering to the drug product label and reviews posted on the FDA website for these drugs.

EXCLUSIVITY PERIOD FOR ENANTIOMER OF PREVIOUSLY APPROVED RACEMATES

It is not required to demonstrate the contribution of each isomer to the effectiveness of the racemic drug being proposed for marketing. Therefore combination drug policy as described in 21 CFR 300.50 is not applied to chiral products. Since combining of the two enantiomers in a racemate drug product is not deliberate, the activities of the enantiomers are usually similar, and in the past the separation was difficult, therefore the combination drug policy is not applied to racemic drugs. However, the mixtures of diastereoisomers are readily separated, and their activities are often very different and therefore are considered as combination drugs and subject to the combination drug policy. At present, marketing exclusivity period for developing a single isomer of previously approved racemate is three years. FDA requested comments (62 FR 2167, January 15, 1997) on the appropriate period of marketing exclusivity for drug products whose active ingredient is a single enantiomer of a racemate that is an active ingredient of a previously approved drug product. Several varied responses were received by the FDA and have been summarized elsewhere [43].

SUMMARY

Stereoisomers is a general term used for molecules that are identical in atomic constitution and bonding, but differ in the orientation of the atoms in space. Literature shows numerous examples of drugs where enantiomers of a racemate show differences in pharmacology, pharmacodynamics, pharmacokinetics, metabolism, toxicity, protein binding, etc. With some

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drugs, one enantiomer may show an entirely different pharmacological response, or may be inactive or less active than the other enantiomer. There may be differences in the degree of toxicity, or different toxic responses may be produced by the pair of enantiomers. When pharmacodynamics and/or pharmacokinetics differences exist between isomers, it can create a significant challenge in interpretation of the activity, if achiral blood level assays are used. Advances in chiral chemistry (manufacturing and analytical) technique have led to a possibility of producing single enantiomer on a commercial scale, and measuring individual isomer levels in biological fluids. The drugs which show enantioselective PK and/or PD add a challenge to the known principles of drug development. For chiral drugs, additional considerations are presented in Table 1 and Fig. 2. In general, for PK assessment of chiral drugs, the main difference (as compared to drug without a chiral center) is the decision whether to use an enantioselective or an achiral assay to characterize the pharmacokinetics.

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A Regulatory View of Liposomal Drug Product Characterization

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INTRODUCTION

Liposomal drug products are defined as drug products containing drug substances (active pharmaceutical ingredients) encapsulated in liposomes [1]. A liposome is a microvesicle composed of a bilayer of lipid amphipathic molecules enclosing an aqueous compartment [1]. Liposome drug products are formed when a liposome is used to encapsulate a drug substance within a lipid bilayer of lipid amphipathic molecules enclosing an aqueous compartment [1]. Liposomal drug products are a relatively new "class" of drugs. Doxil (liposomal doxorubicin), for example, was only approved in late 1995 and there are only a handful of approved products (Ambisome, Abelcet, Amphotec, Daunosome, Depocyt, Doxil), and a limited number of newer products are at various stages of development. As a result, regulatory thinking on these types of products is not as well evolved as it is for more traditional oral or intravenous formulations. However, the Guidances for Industry for orally administered products, and the concepts that underlay them, are also useful guides for our approach to evaluating liposomal

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products [2–4]. Although these agents are generally administered intravenously, they also share many characteristics with peroral drugs, and especially orally administered modified-release (MR) drugs [4]. As with MR drugs, often the purpose of the liposome is to provide slower drug release and provide a more prolonged circulatory life of the active drug molecule. This approach has apparently been successful for Doxil, which may reduce the cardiotoxicity that is usually associated with doxorubicin [5, 6]. Therefore, many of the concepts used to characterize MR oral formulations can be adapted to liposomal formulations.

How these concepts are adapted is the subject of considerable controversy. It is probably no understatement to say that liposomes are subject to a greater number of factors that can affect product performance than oral formulations. Small changes in liposome composition such as the ratio of the lipids, impurities, source of lipids, source of drug substance, and even the time of year for the same source of lipid, can affect the performance of the liposomal product [7]. Because there is only limited experience with these drug products, some aspects of their characterization have not been finalized. The remainder of this chapter describes what issues are considered important for liposomal drug characterization, in comparison to tablets or capsules, and what issues are still evolving.

BIOANALYTICAL ANALYSIS

As with any drug, there is a basic necessity to measure drug concentrations. The methods used to measure plasma concentrations of the active parent and/or metabolites from liposomal drugs are essentially the same as those assays that are used to measure conventional drugs (e.g., HPLC, GC, LC/MS/MS) [8–10]; there are no significant differences in the analytical platforms used. Therefore, the development and validation of an assay for a liposomal drug is same as it is for a more conventional drug. Characterization of the assay is based on the same elements as an assay for a conventional drug (e.g., LLOQ, ULOQ, accuracy, precision, etc.). Therefore, the detailed discussion on analytical method validation in this edition applies equally to liposomal products [11].

The key difference between liposomal and conventional drugs is the liposome. Liposomal drugs, once administered to a patient, give rise to at least two pharmacokinetically/pharmacologically significant species, namely free drug and encapsulated drug. The measurement of total drug alone can produce misleading pharmacokinetic characteristics, because these characteristics are based on both free and encapsulated drugs. This approach is problematic because it is believed that it is free drug which mediates activity, and the development of PK/PD relationships with total

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drug is often unsuccessful in these circumstances. Therefore, it is necessary to measure both free and total drug concentrations during the development of these products (total drug concentrations minus free drug concentrations will equal encapsulated drug concentrations).

The separation of free drug from encapsulated drug is the most critical step analytically, and it seems to generate the greatest difficulty. The separation of free and total drug may be problematic, depending on how fragile the liposome is; the typical methods of separation are sometimes too harsh for a successful separation. The usual methods include centrifugation to separate the liposome from free drug (where the supernatant is analyzed), or some form of filtration (gel filtration, affinity chromatography, etc.) [8]. Double-labeling a liposomal drug with radioactive tracers is a useful way to distinguish between the liposme and the drug, and it is often done to verify the suitability of a separation method. However, this method cannot be used for routine analytical assays because of the need for tracer incorporation, which typically is not a component of the approved drug product. More detailed descriptions of liposomal drug separations are available in the scientific literature.

IN VITRO DRUG RELEASE TEST

In vitro release (IVR) testing is an important component of liposomal drug characterization. These products are typically administered intravenously, and might seem to be exempt from bioavailability or bioequivalence characterization because changes to intravenous formulations generally only require adequate CMC characterization to be acceptable [12]. However, the liposomes are generally used to modify the pharmacokinetic and hence the pharmacodynamic behavior of drugs. Therefore, assessing the characteristic release of the drug from the liposome is crucial. In vitro release is an in vitro characterization of how the liposomal drug performs with respect to release of the active drug moiety. The concept of IVR is similar to a dissolution comparison of oral formulations (tablets and capsules) [3]. In vitro release represents the final test that assesses the effect of all the individual chemical characteristics that can affect the drug product performance. The result serves as a product benchmark, against which future production batches, modified liposomal formulations, and possibly generics (if any such entity can be defined) can be evaluated. This evaluation is important, because IVR is developed with the drug formulation that was used in the clinical phase 3 trials in which the safety and effectiveness of the drug product were evaluated. The IVR is the in vitro standard that is used to assure that production batches of the drug product perform comparably to the clinical trial formulations, and assures the user that the product will

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deliver similar effectiveness and safety as that of the phase 3 trials conducted during product development. In cases where differences are detected, this finding usually indicates the need for an in vivo bioequivalence study to determine whether the products actually differ significantly in vivo.

Once a satisfactory IVR test system is established, the amount of drug released into the solvent is measured as a function of time (see Fig. 1). The rate and extent of drug release measured by this process is a characteristic of the drug product and the specific test system. For oral formulations, a product specification is reviewed and accepted by FDA at the time of drug approval. For example, Q 80% in 15 minutes for a tablet means that not less than 80% of the drug is dissolved and in solution within 15 minutes. All production batches of this tablet are expected to possess this same performance characteristic. Furthermore, the effect of modifications to the tablet formulation in terms of dissolution and solubility should be distinguishable from the original formulation by the dissolution comparison. Small insignificant changes should have no effect, whereas important changes that affect dissolution should be reflected by the dissolution test.

Similar reasoning can be applied to IVR and liposomal drug products. Therefore, the drug developer can approach the IVR in a similar manner. First, the test conditions must be established. The test conditions consist of the apparatus to be used, as well as the solvent, stir rate, temperature, sampling time, and method of quantification. The goal of this test system is to distinguish between liposomal formulations that do and *do not* perform as acceptably as the reference formulation. The development of this test system is more difficult than a dissolution test for a conventional tablet or a capsule. Liposomal performance is sensitive to many seemingly small influences. Small impurities, differences in the source of liposomal material, and temperature are a few of the examples that are known to have had a significant impact on liposomal performance.

For oral formulations, the test system typically consists of a beaker with solvent that is agitated by a paddle at a given rate (USP method 2) [13] (see the chapter on dissolution testing in this edition). Alternatively, a basket rotated at a given rate (USP method 1) is frequently used for capsules [13]. Normally, only some (relatively) minor "tweaking" is necessary before finalizing a method. The FDA and the USP recognize these methods as the "state-of-the-art" methodologies; deviating from these generally prescribed methods requires justification.

However, the development of IVR methods are somewhat more problematic. The release of drug from the liposome is usually dependent upon "sink" conditions that are not easily reproduced in vitro. For example, in the static conditions of a fixed volume of buffer in USP method 2, the drug concentrations equilibrate because of the lack of "sink" conditions.

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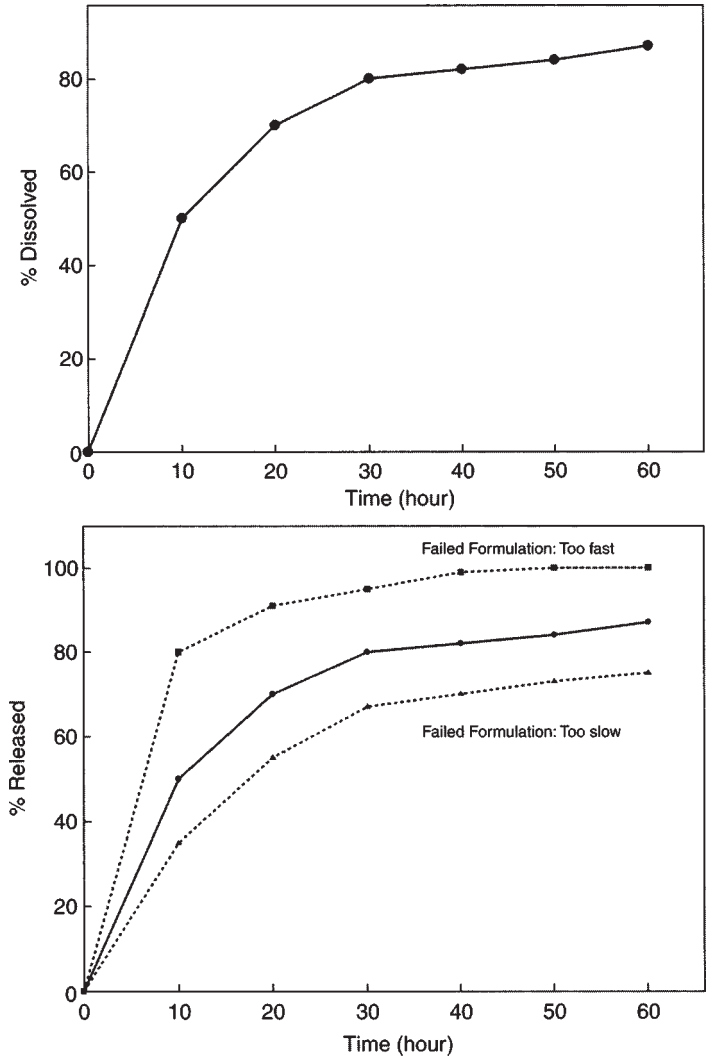

FIGURE 1 A typical dissolution profile for a tablet or capsule is shown (upper panel). An IVR profile for a liposomal drug is shown in the lower panel; Formulations that release drug too quickly and too slowly demonstrate how the IVR should be able to distinguish between good and poorly performing formulations.

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This is probably the single greatest difficulty to overcome. The area of test apparatus for liposomes IVR is currently an area of considerable research. Other approaches, such as membrane diffusion, in situ and continuous flow techniques, have been tested. The continuous flow techniques show considerable promise, as sink conditions are maintained, but there is no clear methodological choice yet. The consequence is that, unlike tablet/capsule dissolution, no standard apparatus is currently available for liposomes.

In terms of sampling, the drug release should be assessed for a period of time that is adequate to characterize 80% of the drug release from the liposome, or until an asymptote is reached [4]. The three batches (two pilot batches and one small-scale batch) which are used for stability testing should also be used for IVR development and product specification. Comparisons should be made using the *f*2 similarity test, as with dissolution, which is currently believed to be the appropriate means for comparing formulations. A difficulty that frequently arises is the time required for the release of 80% of the drug. This final endpoint is often achieved only after days of incubation. This time constraint is problematic for routine monitoring of production lots. Several groups have attempted to address this problem by accelerated IVR designs. These approaches have incorporated changes to the method such as increased temperatures or inclusion of modifiers that accelerate drug release. Although some of these approaches have successfully increased drug release over a more convenient time frame, the relation of accelerated release to actual product performance in vivo is usually unknown. Furthermore, there is currently no consensus on the most appropriate means for addressing this situation, and it too is another area of active investigation. Therefore, these situations are typically dealt with on a case by case basis.

METABOLISM AND PHARMACOKINETICS

Many of the liposomal drug products consist of a previously approved free drug that is encapsulated in a liposome. Often, it is assumed that the metabolic and pharmacokinetic behavior of the liposomal drug is the same as the unencapsulated drug. However, the metabolism and pharmacokinetics of the liposomal drug may be different compared to the free drug [14]. Therefore, it is always advisable to evaluate the metabolism and pharmacokinetics of the active ingredient when encapsulated in liposomes. These studies should therefore compare, where appropriate, the absorption, distribution, metabolism, and excretion (ADME) of a liposomal and nonliposomal drug when

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- the two products have the same active moiety,
- the two products are given by the same route of administration, and
- one of the products is already approved for marketing.

Metabolic characterization should incorporate an in vitro screen (e.g., cytochrome P-450 substrate, inhibition and induction, if the free drug is metabolized by this pathway), and an in vivo study if necessary. Furthermore, in cases where satisfactory mass balance information is available for the free drug, then it is feasible to evaluate only the excretion of the liposomal drug via the major route of elimination. However, if the drug is not approved in another dosage form, then a full mass balance study similar to that for any other new molecular entity that delineates the metabolic pathways and metabolites should be conducted.

It is also important to determine whether encapsulation of an active ingredient into a liposome alters the volume of distribution *(V_d)* and clearance (CL) of the active ingredient. Typically, this alteration of V_d and CL is the purpose of liposomal encapsulation, but occasionally, liposomes have been used to enhance drug solubility. Pharmacokinetic studies should include single-dose and multiple-dose studies that evaluate the pharmacokinetics of the drug substance after administration of the liposomal drug product, and a dose proportionality study over the range of doses that are expected to be used in the patient population.

IN VIVO STABILITY

The stability of a liposome drug product in biological fluid is important for a safe and effective application of the drug product. It is necessary to determine that the integrity of the liposome drug product is not compromized prior to reaching its site of action. Therefore, it is essential that a bioanalytical method that can distinguish between the encapsulated and unencapsulated drug (free) product is available (refer to section Bioanalytical Analysis). Currently, there is considerable discussion as to what constitutes a stable liposome drug product. The questions that need to be addressed are

- What amount of drug release is permissible? and
- Is this drug release dependent on the type of liposome and the intended site of action?

No clear consensus concerning a suitable definition of a stable liposomal drug product has been reached. However, one possible definition of a stable liposomal drug product could be that, if over the time course of the in vivo

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single-dose study, the drug substance remains substantially in the encapsulated form, and the ratio of unencapsulated to encapsulated drug substance remains constant; then the liposomal drug product could be assumed to be stable in vivo [1]. Depending on the intended site of action of the liposome, when the liposome is stable in vivo, the total drug substance concentration could be sufficient to determine the pharmacokinetics and bioavailability of the active pharmaceutical ingredient. However, for an unstable liposomal drug product, the concentration of both encapsulated and uncapsulated drug substance should be determined in evaluating the pharmacokinetics and bioavailability of the drug product [1]. The in vivo stability of the liposomal drug product will also be influenced by protein and lipoprotein binding. Hence, the interaction of proteins and lipoproteins with liposomal drug product should be evaluated.

Drug interaction studies, studies in special populations such as hepatically and renally impaired patients may have to be conducted depending on the metabolic fate of the active pharmaceutical ingredient after encapsulation in liposomes.

BIOAVAILABILITY AND BIOEQUIVALENCE

The important factors in assessing bioavailability and bioequivalence of liposomal drug products are the release of active moiety from drug product and the availability at the site of action. Liposomal drug products either act to deliver drug to a "depot" site from where drug is released slowly into the systemic circulation and then to its site of action. Alternatively the liposomes are intended to deliver the drug to a specific site where the drug acts after release from the liposomal drug product (e.g., tumor uptake of a liposomal drug). Therefore, depending on the type of liposomal drug product, a number of questions arise, such as

- Can it be assumed that the plasma drug concentration is an adequate surrogate for safety and effectiveness of these drug products?
- Should the lipid moiety be considered as a functional excipient?
- Should it therefore also be required that a reformulated product or generic product be quantitatively the same as the innovator in this respect?
- Does the traditional definition of pharmaceutical equivalence apply to liposomal drug products?

Depending on the intended mechanism of delivery of the active pharmaceutical ingredient, it may be feasible to conduct bioequivalence

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studies using pharmacokinetic parameters as endpoints. The critical requirement is the availability of a validated, sensitive analytical method capable of measuring encapsulated and unencapsulated active ingredient. The liposomal drug product must be stable in vivo. For liposomal drug products intended to act as a depot and release the drug slowly in the systemic circulation, it may be feasible to conduct bioequivalence studies between drug products using pharmacokinetic measures as the endpoints. For such products, the regulatory criterion that needs to be fulfilled is that the confidence interval around the ratio of test product to reference product must fall within 80 to 125% for log-transformed AUC and C_{max}.

For liposomal drug products designed to deliver the active ingredient to a specific site, it may not be feasible to conduct bioequivalence studies using pharmacokinetic parameters as endpoints. Other methods stipulated in the CFR for determining bioequivalence, such as comparative clinical safety and efficacy studies, should be considered as a means of evaluating whether the liposomal products are therapeutically equivalent. It must be remembered that these other methods are considered less sensitive in determining the bioequivalence of the two products. Therefore, the sample size and the criteria for determining bioequivalence may be more stringent than a traditional bioequivalence study.

CONCLUSIONS

Generally, the development of liposomal drugs is comparable to traditional formulations, albeit with the need to address certain liposome-specific issues. A sensitive specific assay that characterizes free and encapsulated drug, adequate CMC characterization, and IVR test system development help direct the in vivo development of a liposomal drug product. Good biopharmaceutic characterization underpins the clinical pharmacology characterization of a liposomal drug. Disposition, metabolism, and excretion of liposomal drugs need to be assessed as new molecular entities, although the extent of these studies may be abbreviated. Liposomal drug behavior in special populations may also need to be addressed depending upon metabolism and excretion studies. Bioequivalence studies for altered formulations and generics (if possible) can be conducted according to current practices for free and liposomally encapsulated drugs. It is also advisable to work with regulatory authorities. Periodic contact with regulatory authorities during the development of a liposomal drug product can help to avoid significant differences in expectations regarding the characterization of the drug at the NDA stage.

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Challenges in Drug Development: Biological Agents of Intentional Use

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INTRODUCTION

The U.S. anthrax outbreak of 2001 has demonstrated the possibility that biological agents may be used intentionally to cause human disease. This new awareness underscores the urgency of the public health need for safe and effective medical countermeasures. Attention to the challenges in the development of medical countermeasures against biothreat agents can facilitate their availability. A list of the diseases that can result from the intentional use of the highest threat biological agents is presented below. It is followed by a discussion of special issues in drug development presented by these diseases, and of regulatory mechanisms that can enhance the availability of such drugs. The chapter concludes with examples of recent regulatory actions taken by Food and Drug Administration (FDA) to make available safe and effective drugs for this urgent public health need.

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BIOLOGICAL AGENTS AND ASSOCIATED DISEASES

In June 1999, the Centers for Disease Control and Prevention (CDC) convened a panel of experts to identify the biological agents considered to be of greatest potential concern. The result was three categories of agents. Because they cause high mortality or serious illness and are relatively easy to spread, the organisms in Category A were thought to be of greatest concern. These agents warrant increased surveillance and the availability of appropriate therapy or prophylaxis for diseases caused by them [1].

Biological agents-category A (US CDC, June 1999)

Organism	Disease
Variola major	Smallpox
Bacillus anthracis	Anthrax
Yersinia pestis	Plague
Clostridium botulinum toxin	Botulism
Francisella tularensis	Tularemia
Filoviruses/Arenaviruses	Hemorrhagic fever

ISSUES IN DRUG DEVELOPMENT

The development of efficacy and safety data needed to support the regulatory approval of a drug for an indication related to the intentional use of a biological agent raises a number of issues. Many diseases caused by biologic agents of intentional use rarely occur in nature or are known to contemporary physicians only by historical reputation. Still others, while continued public health problems occur in remote areas of the world where the collection of data and conduct of clinical trials are extremely difficult. It is unethical to introduce any of the agents into a human population for any purpose, including the evaluation of drugs.

Up until 2001, there had been 18 cases of naturally occurring inhalational anthrax reported in the United States, and the events of 2001 resulted in an additional 11 cases [2]. Inhalational anthrax differs from many other infections that result from exposure to biothreat agents in that there was a large outbreak of human disease in Sverdlovsk in the former Soviet Union. This is thought to have resulted from leak at a military research facility, and resulted in at least 66 deaths. After several years, an international team of pathologists published their postmortem findings from these patients, thus expanding the knowledge of the course of this infection in humans [3]. This rather sparse database on human disease is one of the most robust for diseases caused by biothreat agents. Naturally occurring smallpox was declared

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eradicated from the world in 1980, and last seen in the U.S. in 1947 [4]. Few practicing physicians have seen a case. Pneumonic plague occurs naturally, but small foci of disease have been found in remote locations that make systematic study difficult. Intentionally caused disease may differ from what occurs naturally by a number of variables such as inoculum size, route of exposure, number of individuals exposed, and rate at which infection may progress through a population.

There is little regulatory precedent for review of products for such rare diseases. Even for inhalational anthrax, for which there is some body of data on human disease, the database is scant when compared with the hundreds or thousands of patients enrolled in phase III clinical trials of drug evaluation for more common indications. The need to evaluate drug efficacy for such diseases can be met in part by the use of animal models. Recent finalization of the animal efficacy rule [5], which describes the use of animal models for efficacy evaluation of drugs, represents a new direction in regulatory approaches to products for use in patients exposed to biothreat agents. The recognition that there may be scientifically valid animal models from which drug efficacy information can be derived addresses in part the problems presented by the need for systematic study for these rare human diseases. However, access to experimental animals and appropriate laboratory facilities for such studies can be another limiting factor.

REGULATORY MECHANISMS TO ENHANCE PRODUCT AVAILABILITY

The development of drugs as countermeasures to bioterrorism present a number of challenges that heighten the urgency of this public health need. A number of regulatory mechanisms may be used to address this need. They are presented below according to stages of product development.

PreIND Meeting

Prior to the submission of an investigational new drug application (IND), a sponsor may request a preIND meeting with the review division, a means of opening dialogue with FDA. During this period the sponsor may seek guidance regarding a wide range of scientific issues, and the preIND meeting offers an early and systematic way to address them. The process is designed to be efficient, and permits simultaneous review across all relevant scientific disciplines. The preIND meeting provides regulatory guidance early in the development process. It is particularly helpful for drug development that presents special challenges such as those cited for countermeasures for bioterrorism. Dialogue can begin at any time during the preIND phase, and

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may address issues including the leveraging of scarce resources such as experimental animals.

IND Regulations

The IND phase refers to the period that extends from the first use of a product in human subjects up to the approval for marketing. Prior to approval, any product may be considered investigational, including those that are already approved for indications other than that under development. During this phase, drugs may still be made available for clinical use. Such use should be consistent with the IND regulations [6]. There are three basic requirements for the IND use of a drug. These are (1) obtaining informed consent from any patient or subject that receives the drug, (2) using the product under a protocol of planned use that has been reviewed by an Institutional Review Board (IRB), and (3) collecting outcomes data that describe safety and/or efficacy of the investigational product. FDA has recognized the need to maintain a regulatory standard of safety and efficacy while meeting the agency's responsibility to make medical countermeasures readily available in a public health emergency such as a release of a biologic agent. In this regard, sponsors such as federal or local public health agencies may make use of a "streamlined IND" or "contingency protocol" that adheres to regulatory requirements while meeting emergent need. Such applications may be appropriate to a population exposed to a biological agent.

NDA Regulations

The new drug application (NDA) regulations describe the standards of drug approval for marketing. Within the NDA regulations are certain provisions that can enhance availability of medical countermeasures against biothreat agents. These include the accelerated approval regulations and the animal efficacy rule.

The accelerated approval regulations [7] describe the use of a surrogate marker of efficacy thought reasonably likely to offer a benefit of decreased serious morbidity or mortality. The regulations require the collection of postmarketing information to validate the choice of surrogate. Such markers have been used for other classes of drugs such as the antiretrovirals, where the CD4 count was considered a surrogate marker. The accelerated approval regulations were the basis of the FDA approval of the first antimicrobial for an indication related to a biological agent of intentional use, ciprofloxacin for postexposure inhalational anthrax. A more detailed discussion of that approval is presented below.

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Finalized in May 2002, the animal efficacy rule [5] may apply to the study of that a disease cannot be studied in humans because it is extremely rare and/or unethical to introduce the disease into a human population. In such a case, this regulation describes the development of efficacy data in a scientifically valid animal model of the diseases of interest. The animal rule applies only to efficacy data; safety data for any drug evaluated in this manner would be expected to be developed in a human population. A product approval based on the animal rule would also require the collection of outcomes data in the postmarketing period.

Priority Review of New Drug Application

At the time of the NDA submission, drug availability may also be accelerated by a priority review. This is a request made by the drug sponsor at the time of submission, and is generally used for products of special public health significance. Priority review status shortens the time of NDA review to six months.

RECENT REGULATORY ACTIONS ON DRUGS FOR BT/BW INDICATIONS

Ciprofloxacin for PostExposure Inhalational Anthrax

In August 2000, the U.S. Food and Drug Administration (FDA) approved Cipro® (ciprofloxacin hydrochloride) for postexposure inhalational anthrax. This was the first antimicrobial drug approved by FDA for use in an infection due to a biological agent of intentional use.

The study of ciprofloxacin for prevention of inhalational anthrax was performed in a nonhuman primate model, the rhesus macaque. It was planned and conducted by investigators at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) in 1990 at the start of the war in the Persian Gulf. The results demonstrated a significantly improved survival rate for animals that received ciprofloxacin following exposure to aerosolized *B. anthracis* compared to animals that received no antimicrobial. Ciprofloxacin serum concentrations were measured in these animals, and it has been shown that these levels are reached or exceeded in various human populations that receive ciprofloxacin in the doses recommended for this indication. Human serum concentrations could also be correlated with clinical outcome when viewed in the context of in vitro drug susceptibility of *B. anthracis*.

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Ciprofloxacin serum concentrations in humans served as a surrogate endpoint for the efficacy of ciprofloxacin in postexposure inhalational anthrax. As such, the efficacy data in the Cipro® application met the criteria for approval under the accelerated approval regulations.

Since the 1940s, studies of inhalational anthrax had been undertaken in a number of animal species, many in the rhesus macaque. The study of human disease resulting from sporadic industrial exposure and from the 1979 outbreak in Sverdlovsk provided an understanding of inhalational anthrax that demonstrated that the macaque is a relevant animal model of this disease. The applicability of this model was based on data attesting to the similarities in pathogenesis, clinical course, and tissue pathology in rhesus monkeys and humans with inhalational anthrax. Ciprofloxacin had been used widely and has a well-characterized safety profile. There also existed a significant body of pediatric safety data such that the indication was approved for pediatric use as well.

The availability of a suitable animal model for inhalational anthrax, the demonstration of a significant survival advantage in experimental animals that received ciprofloxacin, the use of ciprofloxacin serum concentrations in humans as a surrogate endpoint, the well-established body of safety data for this drug, and the unanimous concurrence of the Anti-Infective Advisory Committee constituted the scientific basis for this approval [8].

Doxycycline and Penicillin for PostExposure Inhalational Anthrax

In November 2001, FDA further expanded the options for the management of patients exposed to aerosolized anthrax spores with the publication of a Federal Register (FR) notice providing scientific data and dosing recommendations for two other drugs already approved for anthrax, doxycycline and penicillin [9]. At the beginning of the U.S. anthrax outbreak of fall 2001, the FDA Center for Drug Evaluation and Research (CDER) recognized the need to expand options for the management of individuals exposed to spores of *B. anthracis*. At that time, there were products in the penicillin and tetracycline classes that were already approved for treatment of anthrax in general, but did not include specific dosing recommendations for postexposure management in the label.

It was also recognized that the USAMRIID animal model of postexposure inhalational anthrax that supported the approval of cipro-floxacin also included cohorts that received doxycycline or penicillin. Both of these drugs, for which there are both innovator and generic products, had been approved for decades and both were characterized by a substantial safety database. Review of pertinent pharmacokinetic and safety data for these drugs suggested that sufficient scientific evidence existed to support the publication of dosing recommendations for these two drugs for the management of individuals

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exposed to aerosolized *B. anthracis*. This information was made available to the public as an FR notice in November 2001, with a simultaneous request for manufacturers of these products to submit labeling supplements to FDA such that this indication and dosing information could be added to the package insert [9].

CONCLUSION

The threat of the intentional use of biological agents presents an urgent public health need. Recognition of the agents of highest threat, the challenges presented by the development of drugs to counter these threats, and the regulatory mechanisms to enhance the availability of such drugs are important tools in our biodefense preparedness.

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The Regulation of Antidotes for Nerve Agent Poisoning

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On February 5, 2003, the U.S. Food and Drug Administration (FDA) approved a New Drug Application (NDA) for the use of pyridostigmine bromide as a pretreatment for poisoning with the nerve agent, soman. This approval was granted under recently adopted regulations that permit the marketing of such treatments on the basis of effectiveness data obtained in animal studies. This chapter will discuss the regulatory and scientific issues raised by these applications generally, as well as those considered for this specific application.

The regulatory and scientific questions raised in the consideration of the standards that must be met by a sponsor wishing to market a treatment for individuals exposed to poisoning by nerve agents are complex and novel. In this chapter, these questions will be identified, and potential answers discussed, in the context of a proposed treatment for poisoning with the nerve agent, soman. While the chapter will be concerned with this specific example, most of the issues raised will be relevant to the consideration of the standards to

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be applied to any application for a proposed treatment for nerve agent poisoning.

REGULATORY ISSUES

In order to understand the regulatory issues that are unique to a consideration of applications for treatments for nerve agent poisoning, it is imperative to have an understanding of the legal standards that must be met by any application for marketing of a new drug. In this chapter, we will focus almost exclusively on the effectiveness standard; while the law also requires that a drug be shown to be safe in use, we will not specifically discuss this requirement.

The Federal Food, Drug, and Cosmetic Act (the Act), the statutory basis for drug approval in the United States, sets out the requirements that must be met before an application for a drug product may be approved. Among other things, the Act requires that a sponsor provide "substantial evidence" of effectiveness that the drug will have the effect described in product labeling. The Act itself provides a definition of substantial evidence as follows:

> …"substantial evidence" means evidence consisting of adequate and well-controlled investigations, including clinical investigations, by experts qualified by scientific training and experience to evaluate the effectiveness of the drug involved, on the basis of which it could fairly and responsibly be concluded by such experts that the drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the labeling or proposed labeling thereof [1].

The critical portion of the definition for our purposes is the requirement for clinical investigations with the drug. The word clinical has traditionally been interpreted to mean human; that is, the Act has traditionally been interpreted to require that a drug be shown to be effective in humans before it may be approved for human use.

Typically, clinical trials that have served as the adequate and well-controlled trials on which approval has been based have demonstrated an effect of the proposed treatment on a relevant measure of clinical performance. For example, drugs to treat patients with seizures are approved on the basis of a showing that they decrease the number of seizures compared to a control group. Similarly, drugs to treat patients with Major Depressive Disorder are approved on the basis of the drug's beneficial effect on a scale that assesses the patient's depressive symptoms compared to a control group. Almost all

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drugs are approved on the basis of a beneficial effect on a symptom or sign that is of obvious relevance to the patient's clinical status.

However, some drugs have been approved on the basis of a drug's beneficial effect on a measure that is not immediately obviously relevant to how the patient feels or to the patient's functioning. These measures are called "surrogate markers," and in those cases in which approval has been based on a beneficial effect on such a surrogate marker, the approval has been based on the Agency's finding that the effect on the surrogate can be taken to imply an effect on a clinical outcome of importance.

For example, the Agency has long approved drugs proposed as treatments for hypertension on the basis of a beneficial effect on blood pressure. Blood pressure is a surrogate marker, because it is a measurement that, in and of itself, is not directly tied to the patient's clinical status or symptoms (unless, of course, it is very low or very high). Another example is the class of cholesterol lowering agents. These drugs are approved on the basis of a beneficial effect on serum cholesterol, a laboratory test that is not directly linked to the patient's clinical status at the time of the test. In both of these examples, the Agency has approved treatments because lowering blood pressure (in patients with hypertension) and lowering cholesterol (in patients with elevated cholesterol) have been shown, over time, to result in a decrease in negative clinical outcomes (strokes, heart attacks, etc.). The value of basing approval in these (and other) cases on an effect on a surrogate is that trials designed to assess the important clinical outcomes (stroke, death, etc.) would need to be of extremely long duration, making them essentially impossible to perform adequately.

In 1992, the regulations (those rules promulgated to interpret the provisions of the Act) were amended to explicitly permit the approval of drugs that have an effect on a surrogate marker that had not been shown to definitively produce a clinical benefit. The new provisions, referred to as Subpart H of the regulations, define the conditions under which such an approval may be granted as follows:

> …on the basis of adequate and well-controlled clinical trials establishing that the drug product has an effect on a surrogate endpoint that is reasonably likely, based on epidemiologic, therapeutic, pathophysiologic, or other evidence, to predict clinical benefit [2].

In 1997, the Act itself was amended to include this specific standard as a basis for approval.

Prior to the 1992 change in the regulations, drugs that were approved based on their effects on surrogate markers were approved on the basis of an effect on surrogate markers that were considered to have been "validated"; that is, proven to predict an actual clinical benefit (as in the case of

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antihypertensives and cholesterol lowering agents). After the 1992 amendments to the regulations, however, the Agency could approve a drug on the basis of an effect on an "unvalidated" surrogate marker; that is, approval could be granted on the basis of an effect on a measurement that had not yet been demonstrated to predict a beneficial effect on a clinical outcome. The amendment did, however, require that the clinical effect of interest be shown in studies completed after approval (and, indeed, it was expected that the studies designed to demonstrate this effect would be going on at the time of approval) [3].

It is important to note that this new provision still required that the showing of the effect on a surrogate marker be made in humans; that is, while in some sense the new requirement could be seen as permitting a "lower" standard of effectiveness to be met in certain circumstances (because an effect on a clinically meaningful outcome need not be shown), this provision did not dispense with the requirement in the Act for a finding in "clinical investigations," that is, the drug must be shown to have a beneficial effect on the surrogate marker in humans. While there has been some discussion about whether or not the source of the evidence on which the conclusion that the proposed surrogate marker is considered reasonably likely to predict the clinical benefit can be exclusively derived in animals, the general view is that it can. However, the effect on the surrogate must be, under the new provisions, shown in humans.

Despite this new standard of approval having been incorporated into the law, the Agency felt that there might be situations in which even this standard might be inadequate to support the approval of certain other products, namely products intended to treat patients who had been the victims of various types of poisonings. Specifically, it was felt that it was important to permit the approval of treatments for these patients, but that adequate and well-controlled studies in humans were not feasible for ethical reasons. That is, it was generally considered unethical to perform studies designed to demonstrate the effectiveness of an antidote to poisoning, because such studies would require that subjects be purposefully exposed to the poison. Given this state of affairs, the Agency adopted regulations that set out the evidence that the Agency might rely upon when considering the approval of applications for proposed antidotes to poisons.

These regulations, referred to as Subpart I and published in the Federal Register on May 31, 2002, set out the following requirements:

1. The proposed treatment is intended to ameliorate or prevent "serious or life-threatening conditions."
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3. The results of these studies must be reasonably likely to predict benefit in humans.
4. Studies in animals will be relied upon only where:
    a. There is a reasonably well-understood pathophysiological mechanism of the toxicity of the poison and its prevention or substantial reduction by the drug.
    b. The effect is shown in multiple animal species, or a single species expected to react in a manner predictive of how humans will respond.
    c. The endpoint in the animal studies is clearly related to the desired outcome in humans, usually mortality or an effect on major morbidity.
    d. Data on the kinetics and pharmacodynamics of the drug, as well as other relevant data, allow the selection of an appropriate dose in humans [4].

It is instructive to further examine these requirements.

First, it is important to note that the regulations explicitly state that they do not apply in those cases in which already existing provisions could be the basis for approval (e.g., subpart H in those cases, for example, in which approval could be based on a drug's effect on a surrogate marker in humans, etc.). This explicit statement embodies the Agency's acknowledgment that the proposed reliance on the results of animal studies, while justifiable and nonviolative of the Act's requirements, should only be reserved for extraordinary circumstances [5].

That the regulations propose a unique approach to drug approval is clear, but some discussion is worthwhile to illuminate some of the fundamental differences underlying this approach and current practice and standards of drug approval.

The notion that a drug may be approved for marketing in humans on the basis of data in nonhuman species highlights an important concept routinely applied in current drug approval.

Ordinarily, a drug is approved for marketing on the basis of an empirical demonstration of benefit on an outcome that is considered self-evidently meaningful to the patients (or, less frequently, as we have seen, on a surrogate measure that predicts such an effect). Critically, the presumed mechanism of action of the drug, while of interest and even importance in certain regards, is of little regulatory concern. That is, a detailed understanding of how the drug produces the effect of interest is not required for drug approval, in the typical case. A sponsor is required to show that the drug is effective (appropriately defined), but is not required to prove the mechanism of its effect. Indeed, it is fair to state that we

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have a complete understanding of the mechanism of action of perhaps only a tiny fraction of currently approved drugs, but the Act does ensure that they have been shown to be effective. Were the Agency to require that a sponsor identify a drug's mechanism of effect prior to approval, few drugs would ever reach the market. The reasons for not requiring a sponsor to document the mechanism of action of a drug prior to its approval are clear: the pathophysiology of most diseases is not completely understood, and therefore it is irrational to expect that all of a drug's relevant actions can be identified at any given time. Further, our current understanding of a disease's pathophysiology and a drug's actions may, ultimately, turn out to be incorrect, and it would be inappropriate to base the approval of a drug product, even in part, on such an incorrect understanding. The law's requirement that the drug be shown, empirically, to be effective, is the most appropriate effectiveness standard that can be applied.

Similarly, typically, current drug approval attempts to rely on the fewest possible assumptions about other aspects of a drug's effects. The Agency, again, ordinarily relies upon an empirical demonstration of effectiveness as provided by data from clinical studies that are adequate and well-controlled (i.e., appropriately designed and conducted), rather than relying on assumptions about underlying pathophysiologic events, presumed mechanism of action of the drug, etc. For example, the requirement for a concurrent placebo control group (where appropriate), rather than a reliance upon assumptions about patients' responses in the absence of treatment, embodies the Agency's preference for an empirical showing of a drug's effectiveness. Many other aspects of adequate trial design incorporate the need for an empirical showing, rather than an assumption-based conclusion, of effectiveness. As a general principle, if data can be adduced to answer a specific question, this is to be preferred to relying upon assumption-based approaches.

As can be seen from an examination of Subpart I, however, while there is a requirement for the generation of evidence (in animals, for example, the requirement that the drug's effect be shown in multiple animal species), the rule permits a drug to be approved on the basis of a number of (ordinarily untestable) assumptions. Specifically, the requirement that the pathophysiology of the poison-induced toxicity and the drug's mechanism of its amelioration be well understood elevates to a primary position a consideration that is, as explained above, ordinarily a matter of little regulatory import. Further, the over-arching principle on which the proposed rule is fundamentally based, the ability to extrapolate from data in animals to conclusions about a drug's effects in humans, must ultimately be seen as an assumption that would ordinarily be considered unprovable. Indeed, the provisions of the rule, as outlined above, exist to

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provide the maximum reassurance that the results seen in animals will apply to humans. Ultimately, however, this evidence can only provide a reasonable likelihood that this is true; it would ordinarily not be expected to provide proof.

Nonetheless, given the limitations (essentially impossibility) of performing adequate and well-controlled trials of proposed antidotes in patients who have been exposed to deadly toxins, and given the desire to develop and make these products available, under an approved NDA, the requirements of Subpart I are comprehensive and appropriate, with the caveats expressed above.

Given this background of the relevant regulatory issues, mechanisms, and concerns, it will be illustrative to examine these issues as they relate to the development of one potential treatment, pyridostigmine, for the treatment of intoxication with one specific nerve agent, soman.

SCIENTIFIC ISSUES

A chemical agent has been defined by the North Atlantic Treaty Organization as, "…a chemical substance intended for use in military operations to kill, seriously injure or incapacitate people because of its physiological effects." Various of these weapons have been used throughout the 20th century (e.g., mustard gas in World War I, nerve gas in Iraq in the 1980s, etc.) [6, 7]. Here, however, we will concentrate on the development of treatments for intoxication with nerve agents, specifically Soman.

Nerve agents are all members of the class of organophosphate compounds, in which class are also included various available pesticides. Nerve agents were first synthesized in Germany before World War II, and include tabun, sarin, cyclosarin, and soman. These agents are liquid and volatile at room temperature, and can enter the body via inhalation and directly through the skin [6].

The primary action of these agents is to phosphorylate acetylcholinesterase (AChE), and thereby irreversibly inactivate it. Acetylcholinesterase is the primary enzyme responsible for hydrolyzing acetylcholine (the primary neurotransmitter at nicotinic and muscarinic receptors), so the net effect of poisoning with nerve agents is an accumulation of acetylcholine at these receptors. Excessive accumulation of acetylcholine at these receptors gives rise to a number of signs and symptoms, depending, of course, on the degree of such accumulation. Symptoms can range from excessive bronchial secretions, rhinorrhea, miosis, blurred vision, abdominal cramping, increased salivation, sweating, and lacrimation, urinary frequency and involuntary urination and/or defecation, and can progress to vomiting, bradycardia, generalized muscle weakness,

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paralysis, pulmonary edema, hypotension, respiratory depression, seizures, coma, and death [7, 8].

While the nerve agents ultimately bind irreversibly to the AChE the nerve agent-AChE complex can be uncoupled by treatment with oximes (such as pralidoxime), but only within a specific period of time, unique to the specific nerve agent. After this period of time, the binding is irreversible. This time-related irreversibility of binding is referred to as "aging" [9].

Several treatments are currently approved for the treatment of organophosphate pesticide poisoning. Specifically, atropine and pralidoxime are approved for the management of patients who have suffered a toxic exposure to organophosphorous or carbamate insecticides. Atropine is a competitive inhibitor of acetylcholine at muscarinic receptors, and can treat the hypersecretion, intestinal cramping, and bronchoconstriction induced by nerve agents.

Pralidoxime is an oxime; as described above it can "reactivate" AChE by splitting apart the nerve agent-AChE complex, thereby regenerating AChE, making it available to hydrolyze acetylcholine at the synapse. As noted above, if sufficient time has passed before the nerve agent-AChE complex is exposed to pralidoxime, the binding becomes irreversible. In the case of soman, this aging process is extremely rapid (several minutes), and thus pralidoxime alone is not considered to be helpful for treating poisoning with this agent [9].

Pyridostigmine, a reversible inhibitor of AChE, with poor penetrance into the central nervous system, which is approved for patients with myasthenia gravis, has been proposed as a treatment for prevention of mortality in patients exposed to nerve agents, in particular soman, in combination with acute treatment with atropine and pralidoxime. Pyridostigmine is not proposed as an acute treatment for soman poisoning; rather, it is proposed as a prophylactic treatment. In theory, pyridostigmine, given in appropriate amounts and at appropriate times, protects the organism by reversibly binding with (some) AChE, preventing the irreversible binding of these AChE molecules with the nerve agent. In time, the AChE-pyridostigmine complex will spontaneously dissociate, and a critical amount of AChE will be available to hydrolyze acetylcholine at the receptor (if the exposure to the nerve agent has been transient). In this scenario, atropine and pralidoxime are still considered necessary for pyridostigmine to be effective [7, 8, 10].

Given these basic facts, it is instructive to examine the evidence available and the issues raised when applying the Agency's proposed criteria for approval of antidotes to the case of pyridostigmine as a proposed treatment for intoxication with soman.

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EVIDENCE OF PYRIDOSTIGMINE'S EFFECTIVENESS IN ANIMALS

First, it is important to briefly describe the evidence on which is based the claim that pyridostigmine protects against soman-induced lethality.

Studies have demonstrated that when monkeys are pretreated with pyridostigmine, then exposed to soman, and then treated with atropine and pralidoxime, they have significantly decreased mortality compared to monkeys similarly treated with atropine and pralidoxime, but not pretreated with pyridostigmine. The effect of the treatment regimen is assessed by an examination of the Protective Ratio (PR), defined as the ratio of the LD50 (the dose of nerve agent required to kill 50% of the animals) after pretreatment with pyridostigmine to the LD50 without pretreatment with pyridostigmine. In monkeys, PRs of >40 have been seen after pretreatment with pyridostigmine, suggesting a large effect of pyridostigmine pretreatment on soman-induced lethality. In guinea pigs, PRs after pretreatment with pyridostigmine were about four times those seen without pretreatment with pyridostigmine, but no such marked increases in PRs with pretreatment compared to those without pretreatment were seen in mice, rats, or rabbits [9].

Explanations of Pyridostigmine's Differential Effect Across Species

Because reliance on animal studies for drug approval presupposes a consistent finding of the treatment across multiple animal species, the lack of a consistent finding across species requires an explanation to justify that the species in which the beneficial finding is seen are more relevant to humans.

One proposed explanation for the differences seen in degree of protection of the various species relates to the view that relative rates of decarbamylation of AChE after carbamylation by pyridostigmine determine the species-specific sensitivities to pyridostigmine, and that the relatively rapid rate of decarbamylation in monkeys, the species in which pyridostigmine is most effective, is closer to that of humans than to other species (the mechanism of pyridostigmine-induced protection is believed to be carbamylation of the active site of AChE; subsequent decarbamylation must occur in order for the enzyme to be functional). Several articles in the literature present results of studies purporting to compare the rates of decarbamylation in various species, but the results are fairly limited, and not all studies documented such differences [11–13]. Further, these studies only evaluated the activity of the enzyme in plasma and red blood cells (RBC), but provide no assurance that relevant species differences are seen

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at the sites of action that would presumably be relevant for protection in humans (e.g., the neuromuscular junction). In addition, these studies did not examine enzyme regeneration rates in vivo, where concentrations of pyridostigmine might be expected to be more complex (e.g., varying over time) than in these assays, where pyridostigmine concentrations were held relatively constant. Finally, even if a correlation could be shown between rate of decarbamylation and sensitivity of species to pyridostigmine protection (or lack of protection), this does not establish that this is a mechanism that is operative in determining protection. It has even been postulated that if decarbamylation is too fast, this might result in a loss of effectiveness, because this might result in AChE that is available for inhibition by soman, if it is still present in sufficient quantities.

Another explanation for species differences in sensitivity to pyridostigmine-induced protection from soman toxicity that has been proposed relates to species differences in carboxylesterase activity.

This enzyme is considered to be important in the detoxification of soman in those species in which it exists. It has been shown that monkeys and humans have little to no carboxylesterase activity, and therefore, if carboxylesterase activity is indeed an important determinant of pyridostigmine-induced protection, it has been postulated that these two species would be expected to respond similarly to pyridostigmine, in contrast to other species which have higher levels of carboxylesterase activity and do not respond well to pyridostigmine.

This hypothesis has been examined in guinea pig, rat, mouse, and rabbit. Appropriate protection was seen, and the degree of protection was much more similar, and greater, in the presence of a carboxylesterase inhibitor (which "created" species that were, in theory, similar in their degree of carboxylesterase activity to humans and monkeys) [14, 15].

However, a number of questions regarding the role of carboxylesterase in determining species-specific sensitivity to pyridostigmine arise.

For example, it is not immediately obvious, in theory, why the degree of carboxylesterase activity should be a determinant of the efficacy of pyridostigmine. Specifically, carboxylesterase decreases the plasma levels of soman, but it should not, theoretically, affect the plasma levels of soman associated with lethality (although the dose of soman necessary to be given to achieve the level associated with lethality should be greater in species with high carboxylesterase activity compared to those with less activity). If this is true, the protective ratio (the ratio of the doses of soman needed to produce an LD50 with and without pyridostigmine pretreatment) should not change. For example, if a species has twice as much carboxylesterase activity as another species, the dose of soman needed to produce the LD50 in the former will be twice as great as in the latter, *in both pyridostigmine-treated, and nonpyridostigmine-treated animals,* thereby yielding the same

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protective ratio in both species, all other things being equal. On the other hand, it has been hypothesized that, in species with high carboxylesterase activity, the ability of carboxylesterase to eliminate soman becomes saturated with increasing soman doses such that plasma levels of soman increase in a nonlinear fashion (i.e., relatively low levels are achieved with doses below the saturation point). In this case, pyridostigmine, even if it had activity in these species, would not significantly increase the protective ratio. (One way of conceptualizing this is that pyridostigmine can increase the LD 50 of soman in all species, but that it is difficult to show an effect on the protective ratio in species which are already protected by an intrinsically high carboxylesterase activity.)

It is also possible that the carboxylesterase inhibitor given in the studies noted above has additional actions that could explain the results. Monkeys were not used in these studies; a lack of effect of the inhibitor on the efficacy of pyridostigmine in this species, which has low carboxylesterase activity, would support the conclusion that the inhibitor potentiated pyridostigmine in the other species by inhibiting carboxylesterase.

In addition to these caveats, it is critical to note that additional mechanisms, aside from inhibition of AChE, may be involved in soman-induced toxicity. For example, recent articles in the literature implicate the NMDA receptor complex as being important in the production of nerve agent-induced seizures [16–18]; other articles document the effects of soman-induced intoxication on brain levels of GABA-ergic, dopaminergic, and cholinergic systems, as well as on IL-1beta levels in rat brain [19]. These investigations suggest the complex number of systems that may mediate soman poisoning, and the complex time-concentration relationships that occur between levels of a host of chemical species (endogenous species and soman) that result in soman-induced injury, and pyridostigmine-induced prevention of injury, all of which may vary among species. While these studies discuss mechanisms of soman-induced brain injury in various species, and pyridostigmine is considered not to cross the blood-brain barrier, they may seem irrelevant to the question of pyridostigmine's effectiveness. However, there is evidence that pyridostigmine does have central effects, thereby raising additional questions about how well the mechanisms of pyridostigmine-induced protection are understood.

In addition, there may be other actions of pyridostigmine, aside from inhibition of AChE, which may contribute to its ability to protect (in animals) against nerve agent toxicity, including alternate (though currently unrecognized) mechanisms that might diminish acetylcholine activity at the neuromuscular junction. Indeed, it is fair to say that the mechanism of action of pyridostigmine as a pretreatment for soman-induced toxicity may not be completely understood, making it impossible to conclude with certainty that (1) the protection it confers on monkeys (and to a lesser extent guinea pigs)

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will be seen in humans, and (2) monkeys represent the most relevant model for human responsiveness.

DOSE CONSIDERATIONS AND DIFFICULTIES IN THE INTERPRETATION OF DRUG EFFECT ON SURROGATE MARKERS

A further critical criterion for relying on animal studies to support a conclusion about the effectiveness of an antidote in humans is that the animal studies must provide a basis for identifying a dose of the antidote in humans that will be effective.

In the case of pyridostigmine pretreatment against soman-induced toxicity, the dose necessary to produce inhibition of cholinesterase in red blood cells (RBC) of between 20–40% has been proposed as the appropriate dose. The Department of Defense has shown that a dose, in humans, of pyridostigmine of 30 mg every eight hours will result in this degree of RBC cholinesterase inhibition throughout most of the dosing interval.

The degree of RBC cholinesterase inhibition as a guide to appropriate dosing in humans had been proposed as a surrogate marker of activity, as defined earlier. That is, it had been proposed that when RBC cholinesterase is inhibited between 20–40%, humans will be protected from soman-induced toxicity [9]. Because the true clinical endpoint (mortality) cannot be studied in adequate clinical studies, the achievement of the desired degree of RBC cholinesterase inhibition had been proposed as a surrogate for the clinical endpoint of interest.

While this surrogate cannot be validated in humans (i.e., we cannot know, definitively, in humans, if this prediction of protection is accurate), the first step in accepting RBC cholinesterase as a useful surrogate in humans would be to validate its predictive effect in animals. That is (because the experiment can be done in animals), it should be theoretically possible to validate in animals that the degree of RBC inhibition proposed as protective in humans is, in fact, predictive of protection in animals.

Experiments have been performed in animals that allow an evaluation of the validity of RBC cholinesterase inhibition with pyridostigmine pretreatment as a surrogate for survival. These experiments measured pyridostigmine-induced RBC cholinesterase inhibition and protection against soman lethality following a range of pyridostigmine doses. The showing of a correlation between enzyme inhibition and survival would give credence to (though would not constitute proof of) the idea that pyridostigmine-induced RBC cholinesterase inhibition is an appropriate choice for a surrogate in humans.

The results of these experiments, however, in general demonstrated no correlation between pyridostigmine-induced RBC cholinesterase inhibition

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and survival. In particular, the monkey studies showed that the effect on survival when the degree of RBC cholinesterase inhibition was 20–40% was equivalent to the effect on survival when the degree of RBC inhibition was essentially equal to that in the control group. This finding strongly suggests that the increase in survival associated with pyridostigmine pretreatment is not directly related to the degree of RBC cholinesterase inhibition. If this is true, choosing a dose that will ensure protection in humans based on achieving a particular degree of RBC inhibition in humans is not supportable, because it is not a valid surrogate (in animals); that is, the degree of RBC inhibition does not predict the outcome of interest (increased survival) [9].

Even if such a correlation between RBC cholinesterase inhibition and increased survival in the animal had been demonstrated, it might still be a misleading surrogate, because we do not know the relationship (in animals, or, of course, in humans) between the degree of RBC cholinesterase inhibition and cholinesterase inhibition (if cholinesterase inhibition is relevant at all) at the site of action presumably responsible for the protection (e.g., the neuromuscular junction).

Indeed, closer consideration suggests that RBC cholinesterase inhibition is, a priori, likely to be an inadequate surrogate (even if cholinesterase inhibition is a relevant mechanism). RBC cholinesterase inhibition is, in this case, likely to be merely reflective of the plasma level of pyridostigmine. However, surrogates are more likely to be "valid" the more they reflect biological processes that are occurring as close as possible to the final "step" in the pathophysiologic chain of events. This is because there may be many events leading to the production of the symptoms of concern. The more the surrogate is reflective of events in the final "steps" of disease production, the more likely an effect on the surrogate will represent an effect on the symptoms of interest; that is, in such a case, it is presumed that the desired drug effect is mediated through the surrogate, "ensuring" that the effect will be seen on the clinical symptoms (it is for this reason that a detailed understanding of the pathophysiology of the disease, and a detailed understanding of the mechanism of action of the drug, contribute to increased confidence that the drug's effect on the surrogate will have the desired effect on the disease; as noted above, of course, such complete knowledge is usually lacking). The further removed from the final "step" the processes measured by the surrogate are, the greater the possibility that the drug's effect is on a pathway that is not (entirely or at all) related to the ultimate outcome of interest. Observing an effect on the "final" pathophysiologic event(s) helps to increase the likelihood that the surrogate actually reflects the steps in the biological processes that are critical to the production of the outcome. It is critical to note, however, that even in the best case (that is, one in which the relevant mechanism of action of the

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drug and the relevant pathophysiologic events are considered to be understood), a **correlation** of the effect on the surrogate with the clinical outcome of interest cannot be considered proof that the effect on the surrogate must predict the desired clinical outcome [20].

In any event, RBC cholinesterase inhibition fails this test, because it is not a measurement of the effect of pyridostigmine on the final events in the pathway leading to soman-induced toxicity. Although it is possible that a useful surrogate could be one that, simply, invariably correlates with the symptom of interest, and in which the drug's effect on the surrogate invariably correlates with, but is not "causally" related to, the desired clinical effect, reliance on such a surrogate would ordinarily be less convincing than reliance on a surrogate of the type described in the previous paragraph.

Indeed, RBC cholinesterase inhibition is not a measure of protection from soman-induced toxicity at all; as noted above, it is merely reflective of pyridostigmine plasma levels. A theoretically better surrogate would be a measure of the degree of pyridostigmine-induced AChE *protection* in the face of soman exposure, because this would be a measure of the proposed mechanism of action. Again, it is critical to recognize that this mechanism is only proposed, and a correlation of the effect on the surrogate and the desired effect on the clinical outcome of interest cannot be considered to constitute proof of the mechanism of disease production or amelioration.

Ideally, because the life-saving action of pyridostigmine is presumed to be mediated through its action at the neuromuscular junction (NMJ), evaluation of the surrogate proposed above should ideally be performed at the NMJ. However, at least in humans, it is obviously not practical to measure the treatment effect on the surrogate at the NMJ in all treated patients. One approach to validating this new surrogate would be to treat animals with pyridostigmine, expose the animals to soman, and then treat with atropine and pralidoxime (essentially repeat the dosing regimens in the experiments previously performed), evaluate the RBC and NMJ for protection of the enzyme, and demonstrate a correlation between the degree of enzyme protection in the RBC, enzyme protection at the NMJ, and survival. If this correlation can be demonstrated, an ex vivo study could be performed in humans.

Specifically, a small cohort of humans could be treated with an appropriate regimen of pyridostigmine, followed by blood collection and a muscle biopsy (preferably of the intercostal muscles, perhaps in a sample of patients undergoing surgery in which these muscles would ordinarily be exposed). These tissues could then be exposed to soman ex vivo, and the correlation between RBC and NMJ enzyme protection could be assessed. If a similar correlation between blood and NMJ enzyme protection could be

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demonstrated in humans as was demonstrated in animals (which would, in this scenario, have been correlated with increased survival), one could have greater confidence that the new surrogate (RBC enzyme protection) might be predictive of human survival. Again, finding such a correlation would not constitute proof of the effect of pyridostigmine pretreatment in humans, but it might be considered potentially predictive.

SUMMARY

The preceding discussion is intended to outline the requirements that might be imposed on any sponsor wishing to bring to market a treatment for nerve agent poisoning. As can be seen, such an endeavor differs in important ways from the development of typical treatments; that is, treatments for naturally occurring diseases which can be adequately studied. Crucially, the approval of treatments for nerve agent poisoning depends upon assumptions about mechanisms of disease production, drug action, and relevance of animal models to humans, considerations that are usually absent from decisions about drug approval in the typical case. These considerations may raise new and important (and potentially unanswerable) questions about the ultimate utility of the treatment in humans. However, this approach is considered reasonable, given the need for such treatments and the impossibility of performing the definitive clinical effectiveness trials.

Indeed, as noted earlier, on February 5, 2003, the application for the use of pyridostigmine bromide as a pretreatment for soman poisoning, in conjunction with acute treatment with atropine and pralidoxime was approved by the FDA. The data provided were considered to have met the requirements of Subpart I. In particular, the treatment clearly was expected to provide a meaningful therapeutic benefit, there were a number of adequate and well-controlled studies in animals, and the animal studies, taken as a whole, were considered reasonably likely to predict a benefit in humans.

Specifically, despite a lack of certainty (a situation anticipated by the rule's requirement for reasonable likelihood), the mechanism of soman's toxicity was considered reasonably well understood, as was the mechanism of pyridostigmine's protective effect. Further, the discrepancy in response across species was considered well explained by the documented relative differences in carboxylesterase, with the marked increase in protective ratios produced by carboxylesterase inhibition in pyridostigmine-pretreated high-carboxylesterase species considered powerful evidence supporting this theory, and permitting the conclusion that humans will respond similarly to monkeys.

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Finally, the human dose was chosen so as to result in plasma levels shown to be associated with protection in the monkey. In addition, the resultant dose was relatively close to a maximum dose considered well tolerated by otherwise normal, healthy adults.

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Bioequivalence Assessment: Approaches, Designs, and Statistical Considerations

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INTRODUCTION

Bioavailability (BA) and bioequivalence (BE) are very closely related. Bioavailability usually focuses on the release of the active ingredient/active moiety from the drug product to one or more sites of action. Bioequivalence focuses primarily on the comparison of the measures of release of the active moiety (drug substance) between two (test and reference) products. Studies based on BE principles are useful during drug development and approval of a new chemical entity drug product during the IND/NDA period to link between various formulations, to examine the effect of various factors on BA of the drug, and to study the pharmacokinetics of the drug. Bioavailability and bioequivalence principles have been discussed exten-sively in Chapters 2, 9, 17, and 19 of this book. Bioequivalence assessment is a dynamic and evolving discipline with complexities. It has evolved significantly during the

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past few years. Significant information on this rapidly evolving field may be found in the literature [1–10].

The discussion in this chapter is limited to data analysis of BE studies and in no way comprehensive. It focuses on BE studies with a pharmacokinetic endpoint (systemic exposure approach) with emphasis on the practical aspects of bioequivalence assessment from a nonstatistician standpoint.

APPROACHES TO BE ASSESSMENT

It is generally acceptable that differences between formulations would be observed due to biological and other variabilities. Thus, it is important to examine the clinical relevance of such observed/estimated differences and to ascertain how much difference would be acceptable from the safety and efficacy standpoint. Furthermore, it is equally important to ascertain the degree of uncertainty from such a study and also the magnitude of uncertainty that would be acceptable if a difference is observed. In order to address these issues/questions, statistical principles and methodologies are applied.

Assessment of BE is a dynamic field in the biopharmaceutic evaluation of product quality. Various approaches have been proposed to assess BE. These are (a) average bioequivalence (ABE) and (b) population bioequivalence (PBE) and individual bioequivalence (IBE). Each approach has various advantages and disadvantages. However, ABE is the generally applied approach as it is widely acceptable to the regulatory authorities for the approval of drug products. For completeness, PBE and IBE approaches are briefly described, but the focus in this chapter will be on ABE. A brief description of various approaches is presented below:

Average Bioequivalence

Average bioequivalence compares the population averages between the test and reference products. It is based on the ratio of average bioavailability measures of the test and reference formulations over all individual subjects/patients in the study population. The details of the ABE criteria have been described [11].

Population and Individual Bioequivalence

These are novel approaches that include comparison of both population means and variances (variability). In theory, the PBE and IBE approaches reflect different objectives of BE testing that may be conducted at various stages of drug development. These PBE and IBE objectives are embodied in the concepts of prescribability and switchability, respectively [11–15]. In

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addition to the population means, while the PBE approach assesses total variability of the BE metrics, the IBE approach focuses on intraindividual variability of the test and reference products, as well as subject-by-formulation interaction. These factors are important considerations for interchangeability of a drug product. In addition, both PBE and IBE criteria can be scaled to the reference variability. This offers an advantage for BE assessment of certain drug products, i.e., highly variable drugs [11]. There has been considerable debate in the literature about the utility of these approaches and these approaches have not been adopted for BE assessment by any regulatory authorities.

CONSIDERATIONS FOR BIOEQUIVALENCE STUDIES

BE Study Designs

Various study designs are available for conducting a BE study. The design depends on the pharmacokinetics of the drug, and the number and type of treatments to be tested. Discussions on designs can be found in the literature (16–27).

Crossover Design

In most cases, a crossover design is preferred. In this design, each subject acts as his/her own control, thus minimizing the variability and increasing the study power. Examples of various crossover designs are presented in Table 2. The following are the two major classes of crossover designs that are used in BE studies.

Nonreplicated Crossover Design. A standard two-treatment, two-period, two-sequence design study is an example of this design. In this design, each treatment is administered no more than once to each subject. Basically, one half of the subjects/patients are treated with one drug (test drug) and the other half is treated with the second drug (comparator or reference drug) during the first period. After an adequate washout period (time required for complete elimination of the drug from the system based on the elimination half-life of the drug), each group of subjects is switched to the other drug in the second period.

In a nonreplicated crossover study other than the standard two-treatment, two-period study, the number of sequences appropriate for the study depends on the number of drug products (treatments) under study. It is usually a good practice that the study design be completely balanced for sequences with respect to the number of subjects.

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TABLE 1 Examples of Dosage Forms for Bioequivalence Assessment

Solid oral dosage forms	Solutions
* Tablets: IR, MR (DR & ER)	* Oral solutions
* Sublingual tablets	* Syrups
* Capsules: IR & ER	* Elixirs
* Powders	Inhalation solutions
Oral suspensions	Nasal sprays (solution formulation)
Parenterals	Nasal sprays (suspension formulation)
* Solutions	Topicals
* Suspensions	* Creams
* Emulsions	* Ointments
Otics	* Gels
* Solutions	* Lotions
* Suspensions	* Emulsions
Ophthalmics	* Swabs
* Solutions	* Solutions
* Suspensions	* Shampoos
Transdermal delivery systems	* Suppositories
Metered dose inhalers	* Enemas

Replicated Crossover Design. Examples of replicated crossover design are presented in Table 2. In this design some of the subjects receive at least one of the treatments more than once. In a standard replicated crossover design study, a single batch or a lot of each of the drug products is dosed twice to the same subject. Each treatment is separated by a washout period.

Recently much attention has been focused on replicate design studies because of their ability to identify subject by treatment interactions (10–15). Replicated single-dose crossover studies have been recommended for modified-release products such as, extended-release dosage forms and transdermal systems that may have different drug-release mechanisms. Furthermore, replicate design studies are often recommended for highly variable drugs where a large number of subjects/patients are needed to achieve adequate study power to demonstrate BE. These study designs are also useful for examining intrasubject variability associated with different treatments, presence and magnitude of subject-by-formulation interactions, and unequal carryover effect of the treatments.

Generally a two-treatment, two-sequence, four-period, replicated crossover design has been used for a BE study while using average BE, individual BE or population BE approaches [11]. However, for regulatory decision making,

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TABLE 2 Some Examples of Nonreplicated and Replicated Study Designs

A = Test 1
B = Reference
C = Test 2
D = Test 3

Nonreplicated crossover designs		Replicated crossover designs	
2-trt x 2-per x 2-seq	3-trt x 3-per x 6-seq	2-trt x 3-per x 2-seq [11]	2-trt x 4-per x 2-seq [11]
A B B A	A B C B C A C A B B A C C B A A C B	A B A B A B	A B A B B A B A
4-trt x 4-per x 4-seq [18]			
A D B C B A C D C B D A D C A B			

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even if a design of BE study is replicate, the acceptable statistical data analyses has to be based on ABE.

Parallel Design

In some cases, such as long-half-life drugs and cytotoxic drugs, there is a concern for using a crossover design. For a long-half-life drug, crossover designs are difficult to conduct, as the study would take a very long time to complete. There is also a strong likelihood of significant subject dropouts, creating problems with study power. In the case of cytotoxic drugs, it is not ethical or appropriate to unnecessarily expose the individuals to the toxic compounds twice. As a result, a parallel design where each group of individuals is simultaneously dosed with a treatment only once is often recommended. However, this design would generally require more subjects than would be required for a crossover design because of inadequate study power considerations.

Sequential Design

Sequential designs are increasingly being used in major clinical trials concerning life-threatening diseases. Most applications have trials designed to establish whether an experimental treatment is superior to a control. However, many trials are conducted with the objective of showing that an experimental treatment is equivalent to a control. Methods have been developed in the context of bioequivalence and appropriate sequential procedures are identified [25–27]. The likelihood of demonstrating bioequivalence when the formulations are truly equivalent depends on the sample size and on the variability of the bioequivalence endpoint. Group sequential bioequivalence testing provides a statistically valid way to accommodate misspecification of variability in designing the trial by allowing for additional observations (which have to be prespecified in the protocol) if a clear decision to accept or reject bioequivalence cannot be reached with the initial set of observation [27].

Study Population

Usually, subjects recruited for in vivo BE studies should be 18 years of age or older and capable of giving informed consent. Bioequivalence studies may be conducted on healthy populations or target (patient) populations depending on the type of drug under study. It is recommended that in vivo BE studies be conducted in individuals representative of the general population, taking age, gender, and race factors into account. If the drug

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product is intended for use in both genders, attempt should be made to include equal numbers of males and females in the study. If the drug product is to be used predominantly in the elderly, attempt should be made to include as many subjects 60 years of age or older as possible. The total number of subjects in the study should provide adequate power for BE demonstration, but it is not expected that there will be sufficient power to draw equivalence conclusions for each subgroup. In such cases, statistical analysis of subgroups is not recommended. Restrictions on admission into the study should generally be based solely on safety considerations. In some instances, it may be useful or even necessary that BE study subjects consisting of the target population for the specific drug. In this situation, attempt should be made to enroll patients whose disease process would be stable for the duration of the BE study.

For the subject selection, inclusion and exclusion criteria should be well defined. Medical history, physical examination, clinical evaluation, and all restrictions (inclusion and exclusion criteria) prior to and during the conduct of the study should be well defined in the protocol and should be strictly adhered to.

Sample Size

This is one of the most important considerations in the assessment of BE. There are important issues to consider while developing a protocol for a BE study, such as (a) how much of a chance or probability of concluding equivalence is desired? (b) what true ratio of test/reference (T/R) averages is one interested in?, and (c) what is the anticipated within-subject coefficient of variation (CV) of the BE metrics?

While developing a protocol for an in vivo BE study, a sufficient number of subjects should be enrolled to achieve adequate study power. Attention should be paid to the possibility of dropouts, add-on subjects, individuals or groups, and replacement subjects. The enrolled subjects should be completely randomized for treatments and sequences. Attempts should be made to assign the same number of subjects to each sequence to make the study balanced. If a multi-center/site/group study is planned, an adequate number of subjects should be enrolled at each site or in each group.

Generally, a minimum number of twelve evaluative subjects may be included in any BE study [11]. When an average BE approach is selected using either nonreplicated or replicated designs, methods appropriate to the study design should be used to estimate sample sizes. Sample sizes for average BE may be obtained using published formulas. The study should have 80 or 90% power to conclude BE between the formulations. Sample size also depends on the magnitude of variability and the design of the study. Variance estimates to determine the number of subjects for a specific drug can be

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obtained from the literature and/or pilot studies. Information on sample size can be found in the literature [28–33].

A sufficient number of subjects should be enrolled in the study to allow for dropouts. Because replacement of subjects during the study could complicate the statistical model and analysis, dropouts generally should not be replaced. If dropouts are to be replaced during the study, the intention should be stated in the protocol *a priori*. The protocol should also state whether samples from replacement subjects would be analyzed even if their data would not be included in the statistical analysis. If the dropout rate is high and sponsors wish to add more subjects, a modification of the statistical analysis may be needed. Additional subjects should not be included after data analysis unless the trial was designed from the beginning as a group sequential design.

BIOEQUIVALENCE CRITERIA

The average BE approach is used to assess bioequivalence for all drug products. Thus, the discussion is predominantly focused on the average BE approach. However, other approaches and criteria have been developed in the recent years. Information on these new approaches and the associated methodologies are available in the literature [11–15].

The general form of the average BE criteria is presented below:

$$\theta_{A1} \leqslant \mu_T - \mu_R \leqslant \theta_{A2},$$

where:
μ_T—population mean for the test product
μ_R—population mean for the reference product
θ_{A1}—lower limit of the confidence interval (0.80)
θ_{A2}—upper limit of the confidence interval (1.25)

Analysis of BE data using the average BE approach focuses first on estimations of the mean difference between test and reference products for the log-transformed BA measure. Subsequently, the general approach is to construct a 90% confidence interval for the difference in the population means and to reach a conclusion of average BE if this confidence interval is contained in the interval. Due to the nature of normal-theory confidence intervals, this is equivalent to carrying out two one-sided tests of hypothesis at the 5% level of significance [34]. The 90% confidence interval for the difference in the means of the log-transformed data is calculated using methods appropriate to the experimental design. The antilogarithm of the confidence limits constitutes the 90% confidence interval for the ratio of the geometric means between the test and reference products.

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STATISTICAL METHODS AND DATA ANALYSIS

Data Processing

Analyses of BE data are typically based on a statistical model for the logarithm of the BE measures. The BE measures are log- transformed generally using natural logarithms. Common logarithms to the base 10 may be used. The choice of natural or common logarithm should be consistent and should be stated in the study report. The limited sample size in a typical BE study precludes a reliable determination of the distribution of the data set. It is not necessary to test for normality of error distribution after logtransformation, or to use normality of error distribution as a reason for carrying out the statistical analysis on the original scale [35]. Logarithm transformation is universally accepted by the national and international biopharmaceutic scientific community and regulatory authorities. However, if there is a need to use data on the original scale, adequate justification should be documented and provided in the study report.

Statistical Methods and Data Analysis

The following discussion focuses on the statistical methods and analysis of data pertaining to the assessment of BE by applying the average BE approach and criteria and using both nonreplicated and replicated crossover study designs. The statistical methods applied to the individual and population BE approach are beyond the scope of this discussion and are not included in this chapter.

Nonreplicated Crossover Designs. For analysis of variance (ANOVA), general linear model procedures (PROC GLM) available in SAS, or equivalent software may be used, although linear mixed-effects model procedures may also be used for analysis of nonreplicated crossover studies.

For example, for a conventional two-treatment, two-period, twosequence (2×2) randomized crossover design, the statistical model typically includes factors accounting for the following sources of variation: sequence, subjects nested within sequences, period, and treatment. The ESTIMATE statement in SAS PROC GLM, or equivalent statement in other software, is used to obtain estimates for the adjusted differences between treatment means and the standard error associated with these differences. A simple example of the codes using SAS version 6 12 for a conventional twotreatment, two-period, two-sequence crossover BE study are presented below:

```
PROC GLM DATA=EXAMPLE;
CLASS SUBJ SEQ PER TRT;
```

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MODEL LAUCT LAUCI LCMAX=SEQ SUBJ(SEQ) PER TRT;
TEST H=SEQ E=SUBJ(SEQ)/HTYPE=3 ETYPE=3;
ESTIMATE "A vs. B" TRT 1–1;
LSMEAN TRT;
RUN;

where:

SUBJ=subject
SEQ=sequence or order of drug administration
PER=period or phase of drug administration
TRT=treatment or formulation or product (A=test, B=reference)
LAUCT=log($AUC_{0\text{-}t}$)
LAUCI=log($AUC_{0\text{-}inf}$)
LCMAX=log(CMAX)

In the case of a nonreplicated crossover design, only one "MODEL" statement is used for all BE measures in ANOVA. The "TEST" statement examines the sequence effect (statistically significant, if $p<0.1$). The output of results using a simulated data set and the above codes as an example is presented in Table 3.

The "ESTIMATE" statement pertains to a two- treatment study design in which the code "A" (test product) precedes "B" (reference product). If the treatments were changed to "T" (test product) and "R" (reference product); the "ESTIMATE" statement would be changed to:

ESTIMATE T vs. R' trt—1 1;

since "R" precedes "T" in the alpha numeric sort order.

Furthermore if there are three treatments, for example, "A" (test product 1), "B" (test product 2), and "C" (reference product), the "ESTIMATE" statements may be changed as follows to estimate differences between products:

ESTIMATE 'A vs. B' trt 1–1 0; (Difference between trt A and trt B)
ESTIMATE 'A vs. C trt 1 0–1; (difference between trt A and trt C)
ESTIMATE 'B vs. C trt 0 1–1; (difference between trt B and trt C)

Replicated Crossover Design. Linear mixed-effects model procedures, available in PROC MIXED in SAS or equivalent software, may be used for the analysis of replicated crossover studies for average BE. The ESTIMATE statement in SAS PROC MIXED, or equivalent statement in other software, is used to obtain estimates for the adjusted differences between treatment means and the standard error associated with these differences. An example of SAS code (version 6.12) statements is presented below [11]:

PROC MIXED DATA=EXAMPLE;
CLASSES SEQ SUBJ PER TRT;

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TABLE 3 The GLM (General Linear Models) Procedure

Dependent variable: [Ln (BE Measure)]

Source	DF	Squares	Sum of Mean Square	*F* Value	Pr > *F*
Model	24	33.73717963	1.40571582	20.83	< 0.0001
Error	21	1.41722176	0.06748675		
Corrected total	45	35.15440138			

R-Square	Coeff Var	Root MSE	1 example Mean
0.959686	3.658706	0.259782	7.100383

Source	DF	Type I SS	Mean Square	*F* Value	Pr > *F*
seq	1	2.00872661	2.00872661	29.76	< 0.0001
subj (seq)	21	31.23031382	1.48715780	22.04	< 0.0001
per	1	0.47907083	0.47907083	7.10	0.0145
trt	1	0.01906837	0.01906837	0.28	0.6006

Source	DF	Type III SS	Mean Square	*F* Value	Pr > *F*
seq	1	2.00872661	2.00872661	29.76	< 0.0001
subj (seq)	21	31.23031382	1.48715780	22.04	< 0.0001
per	1	0.46989801	0.46989801	6.96	0.0154
trt	1	0.01906837	0.01906837	0.28	0.6006

Tests of Hypotheses Using the Type III MS for subj(seq) as an Error Term

Source	DF	Type III SS	Mean Square	*F* Value	Pr > *F*
seq	1	2.00872661	2.00872661	1.35	0.2582

Parameter	Estimate	Standard Error	*t* Value	Pr > |*t*|
Test-reference	− 0.04075855	0.07667808	− 0.53	0.6006

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```
MODEL Y=SEQ PER TRT/DDFM=SATTERTH;
RANDOM TRT/TYPE=FA0(2) SUB=SUBJ G;
REPEATED/GRP=TRT SUB=SUBJ;
ESTIMATE 'A vs. B' TRT 1–1/CL ALPHA=0.1;
RUN;
```

where:
SUBJ=subject
SEQ=sequence or order of drug administration
PER=period or phase of drug administration
TRT=treatment or formulation or product (A=test, B=reference)
Y=LAUCT=log(AUC_{0-t}) or LAUCI=log(AUC_{0-inf}) or LCMAX=log(CMAX)

For analyzing a data set from a replicated crossover design, each BE measure (AUC, CMAX, etc.) is analyzed separately using the above set of SAS codes. Thus the "MODEL" statement specifies one BE measure at a time. An advantage of using a replicated crossover design is that it is possible to determine the estimates of variances associated with betweensubject and within-subject for test and reference products and subject-byformulation interaction. The resultant output from the analysis of a simulated data set as an example from a two-treatment, two-sequence, fourperiod replicated crossover design study is presented in Table 4.

Parallel Design. For parallel designs, analysis of variance using general linear model procedures available in SAS PROC GLM or equivalent software may be used. The statistical model typically includes a factor accounting for only one source of variation—treatment. There are no sources of variation associated with sequence or period as there are no sequences or periods in a parallel design.

```
PROC GLM DATA=EXAMPLE;
CLASS SUBJ TRT;
MODEL LAUCT LAUCI LCMAX=TRT;
ESTIMATE 'A vs. B' TRT 1–1;
LSMEAN TRT;
RUN;
```

The confidence interval for the difference of means in the log scale can be computed using the total between-subject variance.

Estimation of 90% Confidence Interval

Average BE assessment is carried out by determining the 90% confidence interval of the estimate of the difference between the logarithm-transformed means of test and reference BE measures using the two one-sided tests

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TABLE 4

EXAMPLE
A = T (test) B = R (Reference)

Ln (BE Measure)

Average BE Analysis of Replicated Crossover Study

The MIXED Procedure

Class Level Information

Class	Levels	Values
SEQ	2	ABBA BAAB
SUBJ	24	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
PER	4	1 2 3 4
TREAT	2	A B

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	−9.22002189	
1	2	−72.39005073	0.00000000

Convergence criteria met.

G Matrix

Effect	SUBJ	TREAT	Row	COL1	COL2
TREAT	1	R	1	0.25495337	0.18806223
TREAT	1	T	2	0.18806223	0.16576203

Covariance Parameter Estimates (REML)

Cov Parm	Subject	Group	Estimate
FA (1,1)	SUBJ		0.50492908
FA (2,1)	SUBJ		0.37245276
FA (2,2)	SUBJ		0.16444141
DIAG	SUBJ	TREAT R	0.05835992
DIAG	SUBJ	TREAT T	0.06758204

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TABLE 4 Continued

EXAMPLE

Ln (BE Measure)

Average BE Analysis of Replicated Crossover Study

Model Fitting Information for Ln (BE Measure)

Description	Value
Observations	96.0000
Res Log Likelihood	−46.5094
Akaike's Information Criterion	−51.5094
Schwarz's Bayesian Criterion	−57.7590
−2 Res Log Likelihood	93.0189
Null Model LRT Chi-Square	63.1700
Null Model LRT DF	4.0000
Null Model LRT P-Value	0.0000

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
SEQ	1	22	2.12	0.1593
PER	3	53.6	1.69	0.1793
TREAT	1	22	0.24	0.6296

ESTIMATE Statement Results

Parameter	Estimate	Std Error	DF	t	Pr > |t|	Alpha
T vs R	0.03273992	0.06694585	22	0.49	0.6296	0.1

Test/Reference Ratio $= e^{0.03279922} = 1.033$

ESTIMATE Statement Results

	Lower	Upper
	−0.0822	0.1477
Confidence Interval;	$e^{-0.0822} = 0.921$	$e^{0.1477} = 1.159$

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TABLE 4 Continued

EXAMPLE

Ln (BE Measure)

Average BE Analysis of Replicated Crossover Study

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	B	0.70780255	0.10880666	22	6.51	0.0001
TREAT	A	0.74054247	0.09118503	22	8.12	0.0001

Average BE Analysis of Replicated Crossover Study
Estimates From PROC MIXED

Ratio of Geometric Means (T/R): (%)	Lower 90% CI (%)	Upper 90% CI (%)
103.328	92.1073	115.916

procedure [11,34]. The general expression for the test procedure is presented below:

$(\mu_T-\mu_R)\pm t_{\text{edf }(0.95)}$*SE
or,
$(\mu_T-\mu_R)+t_{\text{edf }(0.95)}$*SE (upper confidence limit)
$(\mu_T-\mu_R)-t_{\text{edf }(0.95)}$*SE (lower confidence limit)
or
Estimate+$t_{0.95(\text{edf})}$*SE (upper confidence limit)
Estimate-$t_{0.95(\text{edf})}$*SE (lower confidence limit)

where:

μ_T—population mean for test product

μ_R—population mean for reference product

$t_{0.95(\text{edf})}$—95th percentile of the Student's *t*-distribution for error degrees of freedom from *t* distribution table (p=0.05) or computed from various software packages

edf—degrees of freedom associated with the error term in the result output from the PROC GLM statements

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Estimate—estimate of the difference between test and reference means (geometric) (from the output of the ESTIMATE statement of the PROC GLM code)

SE—standard error of the estimate of the difference between the test and reference product means (from the output of the ESTI-MATE statement of the PROC GLM code)

The antilogarithm of the ESTIMATE gives the test/reference ratio in the normal scale, and the antilogarithm of the confidence limits constitute the 90% confidence interval for the ratio of the geometric means between the test and reference products. An example of the computation of the 90% confidence interval with illustration is presented in Table 5.

The SAS code for the PROC GLM procedure with the "ESTIMATE" statement (at least as of SAS version 6.12) presented above for the nonreplicated crossover design would not calculate the 90% confidence interval. Alternate PROC GLM statements shown below would estimate the 90% confidence interval:

```
PROC GLM DATA = EXAMPLE;
CLASS SUBJ SEQ PER TRT;
MODEL LAUCT LAUCI LCMAX = SEQ SUBJ(SEQ) PER TRT;
```

TABLE 5 Example of Estimation of 90% Confidence Interval using Two One-sided *t*-tests (Estimates and other data taken from Table 3)

Data: EXAMPLE

AUCT:
Least Squares Estimate: -0.04075855
Standard Error of Estimate: 0.07667808
Error degrees of freedom: 21
t-Value for 21 degrees of freedom (from the *t* Distribution table): 1.7207

Estimation:
T/R (Geometric Mean Ratio): $e^{E} = e^{-0.04075855} = 0.960$ or 96.0%
90% Confidence Interval:

Lower limit: $e^{E} * e^{-tSE} = e^{-0.04075855} * e^{(1.7207^{*}\ 0.07667808)}$
$= 0.960^{*}\ 0.876$
$= 0.841$ or 84.1%

Upper Limit: $e^{E} * e^{tSE} = e^{-0.04075855} * e^{(1.7207*0.07667808)}$
$= 0.960 * 1.141$
$= 1.095$ or 109.5%

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```
TEST H = SEQ E = SUBJ(SEQ)/HTYPE = 3 ETYPE = 3;
LSMEANS TRT/PDIFF CL ALPHA = 0.10;
RUN;
```

There may be some disadvantages in using the "LSMEANS" statement instead of the "ESTIMATE" statement. "LSMEANS" may calculate an erroneous difference depending on how the treatments are coded (e.g., R-T instead of T-R). This is due to the alphanumeric sort order used by the SAS. Furthermore, in some cases LSMEANS would be "nonestimable", but the difference between the LSMEANS would be estimable. The "ESTIMATE" statement would give the estimate of the difference between the test and reference least squares means and thus the 90% confidence interval could be estimated. Unlike the PROC GLM procedure, the "ESTIMATE" statement in PROC MIXED procedure shown above for the replicated crossover design would estimate the 90% confidence interval.

ADDITIONAL ISSUES RELATED TO BE STUDY

Add-on Subjects and Group Effect

The BE study protocol should consider the following important factors:

i. appropriate subject inclusion and exclusion criteria,
ii. enrollment of more than the required number of subjects to achieve adequate study power and to compensate for any unexpected dropouts,
iii. randomization of all subjects as a single group before starting the study,
iv. analysis of all study samples at a single analytical site,
v. statistical analysis of data on the BE measures as a single data set.

Generally, BE study designs with add-on subjects are not recommended. However, on many occasions, add-on subjects (groups) are used in a crossover study for a variety of reasons. Examples of study designs with add-on subjects are presented in Table 6. Additional subjects may be enrolled either overlapping the periods of the study or greatly separated in time. Sometimes studies are also conducted at multiple centers, thus additional subjects may be enrolled at different sites at different times. In other cases, for logistical reasons only a limited number of subjects can be studied at one time at a single site, thus creating different groups of subjects.

It is emphasized that there may be considerable risk in using add-on subjects as a discrete group to increase the power of the study after the study has been completed. Using add-on subjects would be like a "second" study with

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TABLE 6 Examples of Study Designs with Different Groups

I

	Period		
	1	2	3
Sequence	1 A	B	
	2 B	A	
		1 A	B
		2 B	A

Add-on subjects during Period 2 of the study

II

	Period		
	1	2	1 2
Sequence	1 A B		A B
	2 B A		B A

Add-on subjects after completion of the original study at the same center

III

	Period					
	1	2	1	2	1	2
Sequence	1 A B		A B		A B	
	2 B A		B A		B A	

Multi-center study

IV

	Period			
	1	2	3	4
Sequence	A	B		
		A	B	
	B	A		
			B	A

Add-on subjects within the conduct of the study

a different group of subjects. Thus, statistical analysis would be carried out to examine whether these two groups of subjects responded differently to the test and reference products (group by treatment interaction). If a statistically significant interaction is detected, it is possible that the data from the two groups cannot be combined to establish bioequivalence. Under those circumstances, the statistical model should be modified to reflect the multigroup nature of the study. In particular, the model should reflect the presence of different groups and the fact that the periods for the first group are different from the periods for the second group. Sometimes the study is carried out in two or more groups and those groups are studied at different clinical sites, or at the same site but separated in time (for example, months apart). Questions may arise as to whether the results from the several groups should be combined in a single analysis.

If one decides to use an add-on study design, the following procedures may be considered:

i. All plasma samples from the two groups should be analyzed at one time. The samples of each subject from two periods should be analyzed together.

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ii. Subjects should be randomized and balanced for treatments and sequences. Thus, in the add-on group there should be an EQUAL number of subjects in each of the two treatment administration sequences (test followed by reference, and reference followed by test), or as close to equal as possible if the number of subjects recruited for the "second study" is an odd number.
iii. The statistical model used to analyze the data should reflect the fact that periods 1 and 2 in the "add-on study" are not the same as periods 1 and 2 in the initial study.
iv. Group by treatment interaction should be examined by appropriate statistical analysis if it is considered that there are two discrete groups. If group by treatment interaction is statistically significant (at the 0.10 level of significance), data from the two groups may or may not be combined depending on several factors. If statistically significant interaction is not detected, this term (the group by interaction term) may be dropped from the statistical model used to compute the 90% confidence interval.

Some examples of the statistical model (in SAS code, version 6.12) for the analysis of variance (ANOVA) to examine the group effect are presented below:

```
PROC GLM DATA = EXAMPLE;
CLASS GRP SUBJ SEQ PER TRT;
MODEL LAUCT LAUCI LCMAX =
GRP
SEQ
SUBJ(SEQ)
PER(GRP)
TRT
GRP*TRT;
TEST H = SEQ E = SUBJ(SEQ)/HTYPE = 3 ETYPE = 3;
ESTIMATE 'A vs. B' TRT 1–1;
LSMEAN TRT;
RUN;
```

An extensive statistical model may be:

```
PROC GLM DATA = EXAMPLE;
CLASS GRP SUBJ SEQ PER TRT;
MODEL LAUCT LAUCI LCMAX =
GRP
SEQ
```

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```
    GRP*SEQ
    SUBJ(SEQ*GRP)
    PER(GRP)
    TRT
    GRP*TRT;
    TEST H = GRP E = SUBJ(SEQ*GRP)/HTPE = 3 ETYPE = 3;
    TEST H = SEQ*GRP E = SUBJ(SEQ*GRP)/HTYPE = 3
    ETYPE = 3;
    TEST H = SEQ E = SUBJ(SEQ*GRP)/HTYPE = 3 ETYPE = 3;
    ESTIMATE 'A vs. B' TRT 1–1;
    LSMEAN TRT;
    RUN;
```

where:
GRP = group
SUBJ = subject
PER = period
SEQ = sequence
TRT = treatment

The "TEST" statements in the second model examine the statistical significance of GROUP, SEQ*GROUP, and SEQ effects (statistically significant if $p < 0.1$). These are supportive information regarding the study. However, GROUP*TRT is the important source of variance.

In the event that GROUP*TRT interaction is not statistically significant ($p>0.1$), this term may be dropped from the model and the data reanalyzed for BE assessment (estimation of confidence interval). An example of SAS code without the GROUP*TRT term in the model is presented below:

```
    PROC GLM DATA = EXAMPLE;
    CLASS GRP SUBJ SEQ PER TRT;
    MODEL LAUCT LAUCI LCMAX =
    SEQ
    SUBJ(SEQ)
    PER(GRP)
    TRT;
    TEST H = SEQ E = SUBJ(SEQ)/HTPE = 3 ETYPE = 3;
    ESTIMATE 'A vs. B' TRT 1–1;
    LSMEAN TRT;
    RUN;
```

On the other hand, if a statistically significant GRP*TRT effect is observed ($p<0.1$), careful consideration should be given as to the appropriateness of combining the data from the two groups. If data should not be combined, BE may be assessed using data from the original/(first) group only.

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Group sequential design, in which the decision to study a second group of subjects is based on the outcome from the first group, calls for different statistical methods and is outside the scope of this discussion. However, the discussions on this design can be found in the literature [25–27].

Outliers

Discussions regarding the outlier issue in BE assessment can be found in literature [36, 37]. On many occasions, discordant or "abnormal" response to the administered treatment is observed for certain subjects as compared to the rest of the study population. There is always a strong tendency to drop those subjects from the data set for BE assessment.

Abnormal response may be considered into the following categories:

Pharmacokinetic Anomaly

This pertains to an unusual value(s) in the drug level in the biological fluid that does not conform to the predicted pharmacokinetic response of that subject at that sampling time. Sometimes the common practice is to reassay only the specific sample(s) in question to confirm the original value or, if appropriate, substitute the original value with the new value generated from the original and reassay values as per the SOP. However, in order to document the reproducibility of the original assay values, it may be prudent to reassay the "suspect" sample(s) along with the "normal" samples of that subject from adjoining sampling times, both earlier and later. Alternatively, plasma samples from the entire treatment for that subject may be reassayed to decide on the substitution of the suspect data. The specific procedure(s) to be followed for the disposition of the pharmacokinetic anomaly must be decided a priori.

Aberrant BE Measure

This is one of the important issues in the assessment of BE. On certain occasions, subject data for one or more BE measures exhibit "suspect" discordant values compared to the rest of the subjects in a study. Because BE studies are usually carried out as crossover studies, the most important type of subject outlier is the within-subject outlier, where one subject or even a few subjects differ notably from the rest of the subjects with respect to a within-subject test-reference comparison. The existence of a subject outlier with no protocol violations could be a manifestation of subject-by-formulation interactions or product failure.

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There may be a tendency to drop the discordant data from the statistical analysis without understanding the probable origin of an aberrant response of that subject. The deletion of that discordant data may have significant impact on the outcome of the study. The clinical protocol for the specific subject(s) may be extensively examined for any protocol violation. In addition, the product quality, such as content uniformity or homogeneity of the testing batch, and the dissolution properties of the product batch in question may be examined as possible cause(s) of this discordant behavior. If no probable cause can be ascertained, the discordant subject is often redosed with a few other study subjects who showed normal response, and their response is redocumented. If the original data were reproduced for the discordant subject, it would show that the original data represent the true response and the subject should not be deleted from the data set. On the other hand, if the redosed data conform to the response of the other subjects in the original study with the observed intersubject variability, it would show that the unexplainable discordant value probably originated at random and there is probably good reason that the discordant data may be dropped from the original data set.

Statistical Outlier

In the past, statistical tests were often applied to identify statistical outliers in the data set. Based on those tests, it was a common practice to drop the discordant data from the data set used for statistical analysis and estimation of the confidence interval. This approach may be unacceptable to some regulatory authorities as it may be due to an underlying, although unidentified reason, instead of being a random occurrence.

Carryover Effect

Carryover (residual) effect is the influence of one treatment administered in a particular period on the response to a treatment in the subsequent period of the study design. Use of crossover designs for BE studies allows each subject to serve as his or her own control to improve the precision of the comparison. One of the assumptions underlying this principle is that carryover effects are either absent or equal for each formulation and preceding formulation. In BE studies it is generally assumed that one only needs to consider first-order carryover effect, i.e., effects that a treatment has on the response to a treatment administered in the next period. However, it is also important to consider the possibility that the carryover effect depends not only on the preceding treatment but also on the treatment being preceded. This is called Direct-by-Carryover interaction. If carryover effects are not equal, then the estimate of difference between the treatment means that is

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obtained without the carryover effects in the model may be biased. The need to consider more than just *simple* first-order carryover effects have been emphasized [38].

In a standard two-formulation, two-period, two-sequence nonreplicated crossover design, the sequence test in the analysis of variance is only available to test for the presence of unequal carryover effects. However, this is a between-subject test, which would be expected to have poor discriminating power in a typical BE study. Furthermore, if the possibility of unequal carryover effects cannot be ruled out, an unbiased estimate of the difference between the test and reference means based on within-subject comparisons cannot be obtained with this design [11].

For most cases of both replicated and nonreplicated crossover designs, the possibility of unequal carryover effects is considered unlikely in a BE study under the following circumstances [17]:

1. The study is single-dose.
2. The drug is not an endogenous entity.
3. An adequate washout period has been allowed between periods of the study, and in the subsequent periods the predose biological matrix samples do not exhibit a detectable drug level in any of the subjects.
4. The study meets all scientific criteria (e.g., it is based on an acceptable study protocol and the matrix samples were assayed using a fully validated methodology).

With respect to a multiple-dose study, it is believed that the possibility of an unequal carryover effect may also be discounted, provided the drug is not an endogenous entity and the study meets all scientific criteria as described above. Under all other circumstances, it is prudent to consider the possibility of unequal carryover effects, including a direct-by-carryover effect.

SUMMARY AND CONCLUSION

Bioequivalence is an evolving applied discipline with various complexities. There have been significant developments in the area of BE assessment in recent years. However, there are several unresolved issues. Novel dosage forms are being developed that will require novel approaches to assess BE. The current approaches and methods applied to the assessment of BE are scientifically sound and dependable. However, due to the limited size of a typical study, every effort should be made to conduct the study with a wellplanned objective and protocol, and established methodologies and controls, so that unbiased data will be obtained to yield reliable results. As a result, conclusions derived from these studies will be scientifically valid and

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reliable. Pharmaceutical scientists and statisticians are making continuous efforts to improve existing methodologies and to develop new methodologies that will possibly require fewer resources and will reduce human testing.

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